UNIVERSITY OF LIVERPOOL

Exploring single trial appetitive conditioning and the modulation of attention

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Jennifer Avril Davies-Owen

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Author's declaration

This thesis is the result of my own work. No part of this work has previously been submitted in support of another application for a degree or qualification at this or any other university or institution of learning.

Frequent Abbreviations

AB	Attentional Blink
ACC	Anterior Cingulate Cortex
BAS	Behavioural Activation System
BIS	Behavioural Inhibition System
BMI	Body Mass Index
BOLD	Blood Oxygen Level Dependent
CR	Conditioned Response
CS	Conditioned Stimulus
CS+	Reinforced Conditioned Stimulus
CS-	Non-reinforced Conditioned Stimulus
DEBQ	Dutch Eating Behaviour Questionnaire
EBA	Emotional Blink of Attention
EEG	Electroencephalogram
ERP	Event Related potential
fMRI	Functional Magnetic Resonance Imaging
FNS	Food Neophobia Scale
GS	Generalisation Stimulus
MTG	Middle Temporal Gyrus
OFC	Orbitofrontal Cortex
RSVP	Rapid Serial Visual Presentation Task (RSVP)
SFG	Superior Frontal Gyrus
STG	Superior Temporal Gyrus
SN	Salience Network
UR	Unconditioned Response
US	Unconditioned Stimulus
VAS	Visual Analogue Scale
VN	Ventral Attention Network

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Abstract

Pavlovian conditioning processes may be central to hedonic overeating. The following experiments were conducted to explore how learning shapes motivational and attentional processes that might enhance reactivity to food-related cues. A primary aim was to explore the efficacy of a novel, naturalistic appetitive conditioning procedure, first described by Blechert et al. (2016), for producing rapid conditioned responses in as little as a single conditioning trial. This novel paradigm was designed to mimic a real-life experience with a new food, from the initial sight, smell and touch, to consumption.

Subjective measures of craving, expectancies and liking tapped in to explicit motivational processes, and an Emotional Blink of Attention (EBA) task, originally reported by Piech, Pastorino & Zald (2010), was used to explore implicit attentional processes. The degree of incentive salience acquired by newly learned cues was measured as the extent to which they captured attention in a rapid serial visual presentation task (RSVP), preventing accurate target detection in a phenomenon known as an attentional blink.

Experiments 1 and 2 (Chapter 3) failed to show evidence for appetitive conditioning on explicit measures, which I suggest can be attributed to the relatively low reward value of the food item chosen for conditioning (marzipan). Findings presented in Chapters 4 – 7 found more convincing evidence that appetitive conditioning shapes motivational processing of newly learned food cues; after a single pairing, an edible object made from a highly rewarding foodstuff (chocolate) elicited cravings, expectancies for chocolate and was perceived as more highly pleasant than a visually similar plastic object. Experiment 4 (Chapter 5) demonstrated that additional trials did not enhance conditioning, supporting the view that single trial learning with hedonic food rewards is a powerful phenomenon. Furthermore, Chapter 6 demonstrated how this learning spreads to cues varying in their similarity to the original conditioned stimulus via generalisation.

A consistent finding throughout Chapters 3-6 was that attentional processing was modulated by this naturalistic conditioning procedure, although not as originally predicted. After just one conditioning trial, both reinforced and non-reinforced cues captured attention more readily than neutral cues, suggesting salience acquisition independent of reward. Whilst Chapter 4 confirmed that reward-paired cues acquire greater salience than novel or familiar cues after a single exposure, it seems that the novelty has a synergistic influence over this process. I suggest that contextual novelty, in the form of the unusual conditioning procedure, promotes further learning and exploration of newly encountered stimuli, thus maximizing the possibility of acquiring reward.

Chapter 7 presents a final experiment, which explored the neural correlates of appetitive conditioning in a single trial. Again, conditioning was evident based on subjective evaluations. Tentative evidence suggested a potential role for the right superior frontal gyrus in enhancing inhibitory control in response to passive viewing of cues signalling no reward. Brain activity in areas related to salience attribution was greater for a reward-paired cue presented briefly in an EBA paradigm. Although, evidence for reward-driven attentional capture was absent at a behavioural level.

Overall, this thesis supports the utility of a novel naturalistic conditioning paradigm for studying appetitive conditioning processes in a single trial. Just one experience with a novel edible object transformed it into a highly desirable, craved cue. Potential applications of these findings for informing treatment and interventions for obesity and eating disorders, as well as methodological considerations and limitations are discussed in chapter 8.

Chapter 1
General Introduction

1.1 Obesity: A global issue

Obesity has risen dramatically over the last thirty years across the globe. According to the World Health Organisation, rates of obesity have almost tripled since 1975 (WHO, 2018). It is predicted that by 2030, approximately half of the world's population will be obese (Finkelstein et al., 2012). Obesity imposes a significant threat to public health, through associations with at least eighteen non-communicable diseases including coronary heart disease, diabetes, mental illness and various cancers (Djalalinia, Qorbani, Peykari & Kelishadi, 2015). Strong associations have also been found between obesity and the prevalence of severe mental illnesses, such as schizophrenia and bi-polar disorder (Bradshaw & Mairs, 2014). Consequently, obesity is a major cause of premature death, and globally more people die from conditions attributable to overweight and obesity than to being underweight (WHO, 2018). The impact of obesity on public health places overwhelming pressure on healthcare systems and incurs a significant economic burden (Tremmel, Gerdtham, Nilsson & Saha, 2017). Therefore, tackling the problem of obesity has become a significant priority for global public health, yet current interventions appear to be falling short.

1.2 Control of energy balance and appetite.

Understanding obesity is essential for developing effective interventions and, at first glance, this would appear straightforward. Control of body weight is presumed to be simply a product of energy balance. If energy intake exceeds energy expenditure then an individual will be in positive energy balance and will gain weight (Hill, Wyatt & Peters, 2012). The traditional mantra of 'eat less, move more' should be more than sufficient to keep weight under control. In support of this, there is a wealth of evidence demonstrating the effectiveness of caloric restriction for significant weight loss in the short term (e.g., Harvey-Berino, 1998). However, obesity rates have

continued to rise and dietary therapies for obesity typically fail (Mark, 2006). It is estimated that 5 years after successful weight loss through dietary interventions and lifestyle changes, 50% of obese dieters regain all the weight they managed to lose (Sarwer, von Sydow Green, Vetter & Wadden, 2009). It is clear that while weight loss might be relatively simple to achieve in the short term, maintaining a healthy body weight long term is extremely complex for many individuals. Current thinking suggests that obesity is multifactorial in nature (Grundy, 1998), with no one factor able to fully account for a person's dietary choices and body weight. When considering mechanisms which control the balance between calories in and energy expended, traditional theories of appetite control posited that there are two parallel processes: homeostatic and hedonic (Johnson, 2013). This model has recently been updated to reflect the contributions of homeostatic, reward and cognitive process, and acknowledges the complex interplay between the three processes (Higgs et al., 2017) (see figure 1.1).

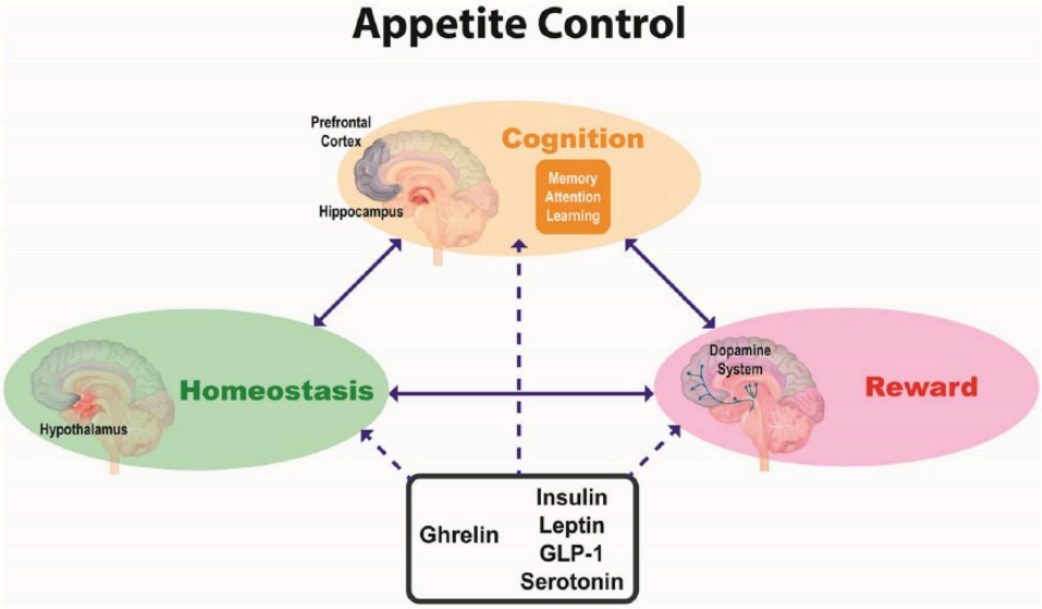


Figure 1.1 A schematic diagram showing the integration of homeostatic, reward and cognitive processes in the control of appetite and eating behaviour. Reproduced from Higgs et al. (2017).

1.2.1 Homeostatic control of food intake

Homeostasis is a biological process whereby an organism's internal state is controlled in order to maintain stable conditions over time (Torday, 2015). For

example, body temperature remains largely stable despite wide variation in environmental conditions. Energy balance has been argued to be under homeostatic control; food intake being determined by an individual's internal state and metabolic need. The hypothalamus has been identified as a key brain area which monitors and affects energy intake and expenditure via a complex interplay of hormones, neuropeptides and signalling pathways (Gao & Horvath, 2008). Any deviations from the norm should result in action; increased adiposity will trigger a reduction in food intake, whereas a state of hunger will elicit eating, in attempts to maintain a stable internal environment (Woods & Ramsay, 2011).

A range of genes have been identified which significantly contribute to obesity via their influence over these internal processes (Comuzzie & Allison, 1998). Research has identified at least 58 loci known to contribute to polygenic obesity (Choquet, & Meyre, 2011), such as polymorphisms of leptin receptor genes (Hastuti, Zukhrufia, Padwaswari, Nuraini & Sadewa, 2016), suggesting that certain disruptions to the homeostatic control of energy balance can lead to obesity. However, it's estimated that genetics can only account for approximately 20% of the observed variation in body mass index (Locke et al., 2015).

Furthermore, contrary to predictions by those in favour of a homeostatic explanation of obesity, food intake appears to be driven largely by external factors. For example, consumption increases as portion size increases, regardless of hunger (e.g., Rolls, Roe, Kral, Meengs & Wall, 2004; Levitsky & Youn, 2004), and humans do not appear to reduce consumption in order to compensate for periods of overeating (Levitsky, Obarzanek, Mrdjenovic, Strupp, 2005).

Much of the recent research into appetite regulation and obesity has focused on homeostatic control of food intake through physiological mechanisms, yet it is clear that this offers an inadequate explanation for obesity and has failed to inform successful obesity treatments. Despite a few rare diseases, such as Prader-Willi syndrome, which causes extreme overeating and weight gain due to hyperphagia (Butler, 2011), failure of homeostatic mechanisms is generally unable to account for the rapidly rising levels of obesity witnessed across the twenty first century. Thus, attention has turned to the environment instead, in an attempt to understand the human drive to eat, beyond metabolic need.

1.2.2 Reward processes involved in eating behaviour

If food intake was determined solely by homeostatic processes, obesity would not be the global problem it is today. Eating would be determined by current physiological need, with little consideration for the taste or pleasure gained from eating. Food consumption would be a necessity, but of little interest to us; similar to our relationship with breathing oxygen. However, our relationship with food is extremely complex and not simply a response to biological hunger. Food is widely used to communicate love and support (Hamburg, Finkenauer & Schuengel, 2014), a delicious bar of chocolate can lift us out of a bad mood (Macht & Mueller, 2007), and a meal at a restaurant is a typical choice for a family celebration. The average UK household spends around £49.60 per week dining at restaurants (Office for National Statistics, 2018); a clear demonstration of how much value is placed on good food. Food brings an immense amount of pleasure; decisions about what to eat, when to eat and how much to consume are not based on carefully regulated internal systems, but often external factors such as the palatability and availability of the food. The anticipation, or experience of pleasure associated with palatable food can lead to excess consumption well beyond metabolic need. This hedonic overeating, Finlayson (2017) argues, is inevitable in our current food environment which promotes excess.

Brown (2012) proposes that such overeating evolved as a means of survival; humans have undergone distinct metabolic changes supporting evolutionary fitness. For example, humans require a near constant supply of energy to meet the high demands of the energy hungry brain, whilst harsh weather, scarce food availability and seasonality associated with the rise of agriculture all added to the pressure to overconsume and store excess fat when the opportunity arose. Evidence from neuroscience tells us that the limbic system and cortex, responsible for emotions and cognition, can easily override the hypothalamus in making food consumption decisions (Berthoud, 2006) promoting hedonic eating over homeostatic control. Schwartz (2001) termed this failure of homeostatic mechanisms, in favour of potent external drivers, the ‘non-regulation’ of food intake, and proposed that weight control may be impossible in the current food environment without daily monitoring of weight and caloric intake. What was once an important survival mechanism, now

poses a significant challenge for humans struggling to resist the lure of calorific foods in our tempting food environment.

In line with the updated three-factor model of appetite control (Higgs et al., 2017), there is evidence to suggest that homeostatic and reward processes interact, rather than being distinct systems. For example, in a phenomenon known as alliesthesia, food becomes less liked and less desirable once an individual is satiated (Cabanac, 1971).

1.2.2.1 Understanding reward: liking and wanting

Recently, the hedonic, or reward value of food has been conceptualised as three distinct yet complementary processes: liking (hedonic pleasure), wanting (incentive salience) and learning (predictive associations and cognitions; Berridge, Robinson & Aldridge, 2009). Elevated levels of both 'liking' and 'wanting' have been implicated in obesity and disordered eating (Finlayson & Dalton, 2012), so any attempts to understand and address problematic eating behaviour should surely address these concepts.

The term liking describes the sensory pleasure derived from food. Most humans can easily recall the enjoyment experienced when eating a delicious food item, such as our favourite flavour of ice cream on a hot summer day. In everyday language, the word liking is typically used to describe this conscious experience of pleasure. Indeed, subjective reports may provide great insight in to the reward value of a food or beverage, perhaps even more so than implicit physiological responses (Beyts et al., 2017). So, it is clear that liking does occur at an explicit, conscious level, as an evaluative process. However, there are also innate neural mechanisms that programme us to respond positively to certain properties of ingesta, and encourage ingestion of nutritionally or energetically advantageous foods. Thus, even in neonates, objective facial expressions of pleasure in response to sweet tastes can be universally observed (Steiner, 1979), and affective responses to subliminal stimuli can occur despite processing occurring outside of conscious awareness (Winkielman, Berridge & Wilbarger, 2005). Thus 'liking', as a specific psychological construct, is used to describe these core hedonic reactions, which may or may not be accompanied by the conscious experience of liking.

A number of localised brain regions, known as hedonic hotspots have been identified, which are able to amplify the 'liking' of pleasant tastes, whereas cold spots can downregulate 'liking' (Peciña, Smith & Berridge, 2006). Hedonic hotspots have been found in the nucleus accumbens, ventral pallidum and brainstem, and more recently it has been suggested they may exist in the orbitofrontal cortex and the insula as well (Berridge & Kringlebach, 2015). Primarily the opioid neurotransmitter system, but also the endocannabinoid and GABA-benzodiazepine systems are responsible for the action of these hedonic hotspots. For example, when microinjections of the mu opioid receptor agonist DAMGO are given in to hedonic hotspot in the nucleus accumbens, rats 'liking' for a sucrose solution increases threefold (Peciña & Berridge, 2005).

However, it seems we are not solely driven by liking: eating can also be driven by desire, cravings or a compulsive need to eat, independent from sensory properties of a food (Finlayson & Dalton, 2012). Again, wanting can mean either the lay term, associated with the conscious experience of desire, or the neurobiological term 'wanting' (Peciña, Smith & Berridge, 2006), where reward acquires incentive salience which determines its motivational value. It is this high incentive salience that can overcome satiety and promote overconsumption of desserts and other palatable foods. Incentive salience of rewarding stimuli appears to be mediated by dopamine release in the mesocorticolimbic pathway, as opposed to the opioid system involved in 'liking' (Berridge, 2007). If incentive salience becomes excessive, a reward may lead an organism to 'want' it even if they do not consciously desire it or even like it. This excessive incentive salience may interfere with the individual's current goals. For example, they may find themselves consuming a forbidden food, despite having clear weight loss goals and consciously deciding to limit their food intake. According to the incentive sensitisation hypothesis (Robinson & Berridge, 1993), this process plays a key role in the development of addictions and addictive behaviour. Through repeated drug use mesolimbic dopamine systems become sensitised to this reward, triggering excessive 'wanting' and further drug taking. However, it has since been demonstrated that this process goes beyond drug use, and applies to many other behavioural addictions such as food, gambling or sex (e.g. Martin & Petry, 2005; Rømer Thomsen, Fjorback, Møller & Lou, 2014).

1.2.3 Cognitive processes involved in eating behaviour

According to Higgs et al. (2017), eating behaviour is modulated by higher-level cognitive processes including memory, attention and learning. When deciding whether to eat a piece of chocolate cake or an apple, long-term dieting goals can guide behaviour towards the healthier choice. However, learned associations between the taste of chocolate cake and the positive hedonic response evoked by eating it may undermine these goals. In addition, holding food-related information (e.g. thinking about the delicious taste of chocolate cake) in working memory may bias attention towards relevant food cues (e.g. the bakery counter at the supermarket), enhancing action readiness and making consumption more likely (Higgs et al., 2012). These mental processes can influence food intake right before eating is initiated, immediately after an eating episode, or in the interval between meals.

Recent evidence has emerged which highlights how cognition can interact with reward in the control of eating behaviour. Asking participants to mentally rehearse which aspects of a meal are most enjoyable can increase food liking, enhancing positive memories about the meal and increasing later consumption (Robinson, Blissett & Higgs, 2012). Similarly, cognitive processes interact with homeostatic mechanisms to guide food intake. Deficits in episodic memory are associated with uncontrolled eating due to poor sensitivity to satiety signals (Higgs & Spetter, 2018). Furthermore, Higgs et al. (2017) identifies several metabolic signalling pathways, which can influence cognition, although more research is needed to determine the exact mechanisms.

The present thesis is primarily concerned with the interactions between reward and two cognitive processes, attention and learning, which are both discussed in depth below.

1.3 Learning about reward

Many rewards innately have high incentive salience; sugars are intrinsically pleasurable and a natural preference for sweet tastes is present from birth and does not have to be learned. However, it has been postulated that certain cues can also acquire incentive salience through their associations with reward (Berridge, 2009). Reward-paired cues can then trigger ‘wanting’, perhaps eliciting cravings and approach motivated behaviour.

Through learning, organisms can use past experiences to make predictions regarding the relationship between the cue and the reward which drive future decisions and behaviour (Berridge, Robinson & Aldridge, 2009). This learning can be explicit in nature and involve the cognitive aspects of reward, allowing the individual to generate expectancies. For example, after eating at a particularly good restaurant, an individual may learn from this experience and decide to eat there again in the future. Crucially, with this type of learning, they will be able to verbalise their reasoning and explain why they have come to that decision. Learning may also be associative and can occur when associations form between a stimulus and reward (classical/Pavlovian conditioning), or between a behaviour and reward (instrumental/operant conditioning). This thesis will focus primarily on Pavlovian conditioning processes.

1.4 Appetitive conditioning.

The ability to learn about the world around us and update our knowledge based on new experiences is an essential survival skill for any organism. Whilst aversive conditioning leads to avoidance behaviour, thus protecting an organism from danger, an organism must also be able to predict the occurrence of appetitive stimuli such as food and water. Appetitive conditioning refers to such a learning process through which otherwise neutral stimuli acquire motivational salience through pairings with pleasant or rewarding stimuli. Aversive conditioning has been extensively studied in both animals and humans using a variety of reliable USs such as electric shocks or aversive tones (e.g., Büchel, Morris, Dolan & Friston, 1998). By contrast, appetitive conditioning in humans is scarcely studied, despite its equally high relevance to survival. This form of learning can encompass several different subsets of conditioning including Pavlovian, operant and evaluative conditioning, which will be described here briefly. As this thesis is primarily concerned with Pavlovian conditioning, its associated processes will be discussed in more depth.

1.4.1 Major forms of learning

1.4.1.1 Pavlovian conditioning

Pavlovian (also known as classical) conditioning was first described by Ivan Pavlov (1927). It is a form of stimulus-response association learning, in which an initially

neutral cue is paired with an appetitive (e.g., food) or aversive (e.g., electric shock) stimulus (unconditioned stimulus; US) (see Figure 1.2). The US always produces some form of unconditioned response (UR) without any learning taking place. According to Pavlov, an unconditioned stimulus should be innate and physiological in nature, without the requirement of prior learning for a response. For example, a rat will automatically demonstrate a fear response to an electric shock even if it has never experienced one before (Silva, Gross & Gräff, 2016), whilst a neonate will demonstrate a preference for previously unexperienced sweet taste (Desor, Maller, & Turner, 1977).

When a neutral cue and US are presented in close temporal proximity, the initially neutral cue comes to predict the occurrence of the US, and becomes a conditioned stimulus (CS). The CS is then able to elicit a conditioned response (CR) similar to the UR, even when the US is no longer present. For example, in Pavlov's classic experiments dogs would salivate (UR) in response to the sight of food (US). A bell was rung each time the dogs were presented with food, and over time the bell became associated with the sight of food. The dogs began to salivate (CR) in response to the bell (CS) even when they could no longer see the food.

According to commonly accepted theories, such as the Rescorla-Wagner model (Rescorla & Wagner, 1972), the strength of the relationship between the CS and the US is determined by the predictive relationship between them. Through CS-US pairings, the CS becomes a 'signal' for the CS, and through conscious awareness of this predictive relationship the individual is now able to generate expectancies regarding the occurrence of the predicted outcome (Martin-Soelsch, Linthicum & Ernst, 2007).

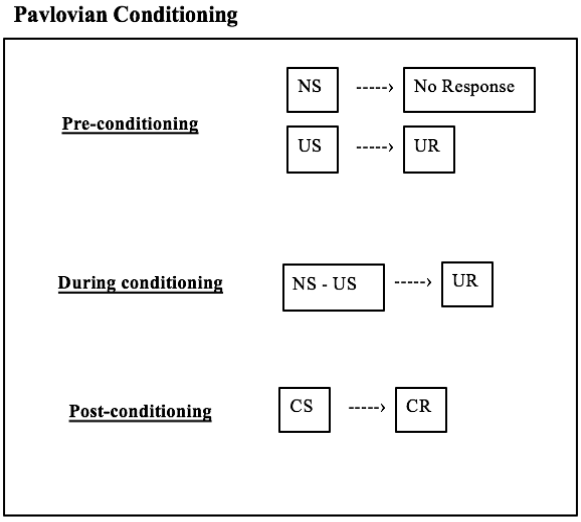


Figure 1.2 Simple depiction of Pavlovian conditioning. Through pairings with an unconditioned stimulus (US), a neutral stimulus (NS) will elicit a conditioned response – the same response as the unconditioned response (UR) produced by the US.

1.4.1.2 Operant conditioning

Operant or instrumental conditioning is a form of response-outcome learning, where an organism learns to associate a specific response or action with a consequence (Skinner, 1938). Reinforcers increase the likelihood of a behaviour being repeated either through the acquisition of something pleasant or removal/avoidance of an aversive factor (see Figure 1.3). Punishment decreases the likelihood of a behaviour either by the addition of something painful or unpleasant, or the removal of something valued. The terms positive or negative precede the terms reinforcement or punishment and refer to the addition or removal of a stimulus, respectively, not their associated valence.

Operant conditioning

	Reinforcer	**Punishment**
Positive (addition of a stimulus)	Increase behaviour	Decrease behaviour
Negative (removal of a stimulus)	Increase behaviour	Decrease behaviour

Figure 1.3 In operant conditioning, an outcome (e.g., food pellet) is dependent on a behavioural response (e.g., lever press). The behaviour can result in either the addition of a stimulus (positive) or the removal of a stimulus (negative) and can either increase (reinforcer) or decrease (punishment) the likelihood of a behaviour.

1.4.1.3 Evaluative conditioning

Evaluative conditioning is a subset of Pavlovian conditioning where the pairing of a US results in a change in valence to the CS, and is considered an important mechanism in the formation of likes and dislikes (De Houwer, Thomas & Baeyens, 2001). Unlike Pavlovian conditioning, evaluative conditioning results in changes in attitude or general affectivity, rather than a biologically relevant response or reflex. For example, in Pavlovian conditioning the Coca-Cola brand name (CS) is paired with the ingestion of the sweet palatable beverage which will result in consummatory reactions such as cravings and salivation (CR). However, an example of evaluative conditioning would be the pairing of the Coca-Cola brand (CS) with photographs of happy people (US). This positive valence should transfer to the Coca-Cola brand and increase preference for it (CV).

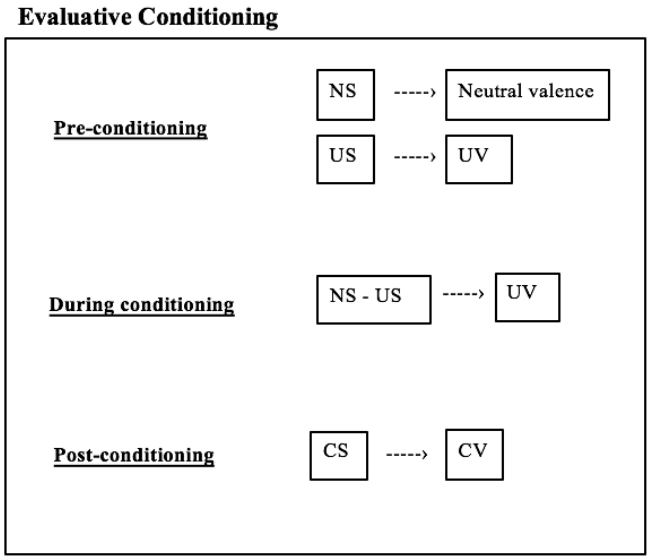


Figure 1.4 In evaluative conditioning an initially neutral stimulus (NS) becomes associated with an unconditioned stimulus (US) with a particular valence (UV). The NS then becomes a conditioned stimulus (CS) and acquires conditioned valence (CV).

1.4.2 Processes and features of Pavlovian conditioning

1.4.2.1 Pavlovian secondary conditioning

Whilst primary Pavlovian conditioning has been extensively studied, secondary, or higher-order conditioning has received considerably less attention. In this form of learning a second CS (CS_2) acquires associative strength by being paired with

another CS (CS_1). Whilst the original CS was directly associated with a US, the CS_2 never was, yet can still elicit a similar CR (see Figure 1.3). Whilst secondary conditioning is inherently weaker than primary CS-US associations, the strength can be maximised if the original conditioning used a particularly high value US (Gewirtz & Davis, 2000).

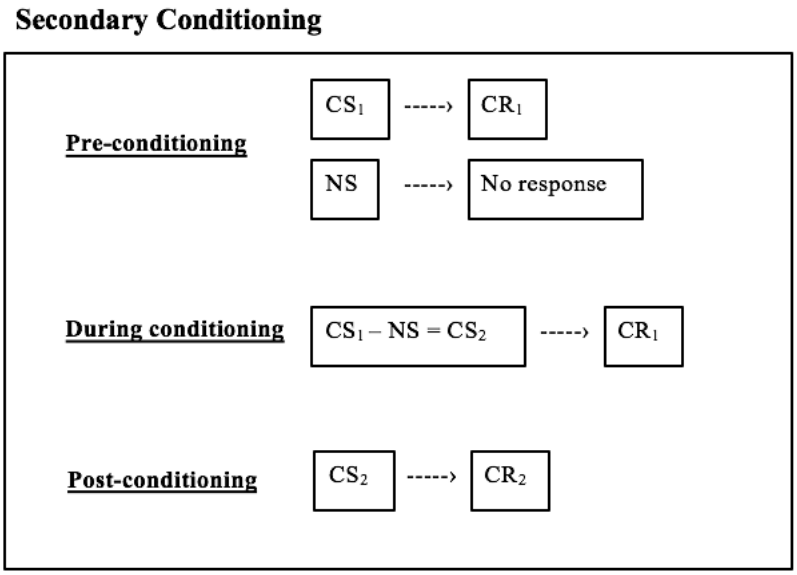


Figure 1.5 In secondary conditioning a neutral stimulus (CS_2) elicits a CR after being paired with another conditioned stimulus (CS_1)

1.4.2.2 Stimulus discrimination and generalisation

In order to respond appropriately and adaptively, an organism must be able to learn to respond to predictive signals efficiently with minimal mistakes. The time costs associated with learning each individual variant of a stimulus would be too great, so they must learn to easily recognise similar stimuli which also signal an appetitive outcome – as well as ignore those that do not (Cerutti, 2001). Two processes are essential for this to occur: stimulus discrimination (Figure 1.6a) and stimulus generalisation (Figure 1.6b).

In a differential appetitive conditioning paradigm, discrimination can be easily demonstrated. A neutral stimulus (NS) paired with a US (CS+) then elicits a CR, whilst a second NS which has no associations with a US (CS-) continues to elicit

no response, as the organism has learned to effectively discriminate between the two stimuli.

Conversely, stimulus generalisation provides an explanation for how learning might spread to other similar stimuli, thus reducing the time course and complexity of learning. A conditioned association can spread from a CS to other neutral stimuli (GS) which have not been directly paired with a US. The GS then elicit a CR in much the same way as the original CS. For example, if a person always watches the news whilst eating breakfast, then watching the news may come to elicit conditioned hunger, cravings and salivation. However, this learning may spread so that other similar cues, such as a different television programme or hearing the news on the radio, will also elicit these responses.

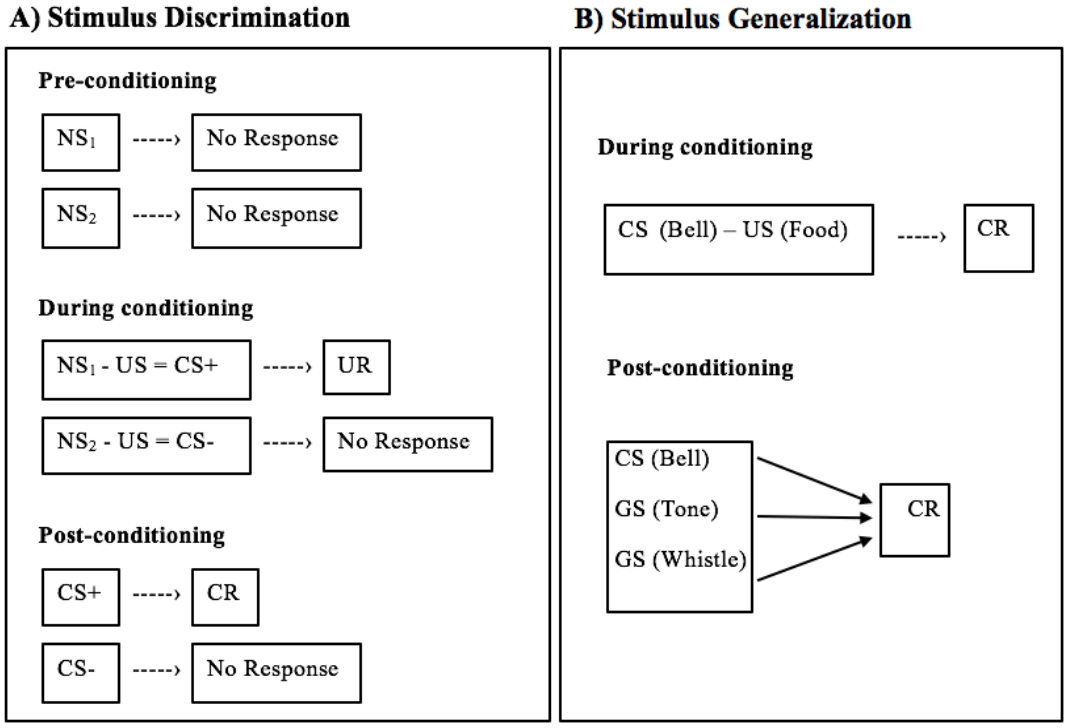


Figure 1.6 A) In discriminative conditioning, a neutral stimulus (NS) becomes associated with the occurrence of an unconditioned stimulus (US). After conditioning, this conditioned stimulus (CS+) elicits a conditioned response (CR). Another neutral stimulus never paired with a US (CS-) does not elicit a response. B) In generalisation learning a stimulus which shares some similarity to the CS (GS) can also elicit a CR despite never having been paired with a US.

1.4.3 Factors affecting the strength of Pavlovian conditioning

Although Pavlovian conditioning is a relatively simple process, multiple factors can influence the acquisition of conditioned responses and their subsequent maintenance. One of the most widely supported factors is the intensity of the CS and US. With higher intensity stimuli, conditioned responses are acquired more rapidly (Passey & Possenti, 1956) and CR magnitude is consistently greater (Beck, 1963; Gray, 1965). The relative lack of research investigating appetitive, relative to aversive conditioning may suggest an inherent difficulty in designing successful appetitive conditioning paradigms, potentially due to challenges in selecting suitable appetitive USs.

Similarly, it is important to carefully consider the value of an appetitive US when designing appetitive conditioning paradigms. Selection of a US is of critical importance: a lettuce leaf may be valued considerably less than a delicious spoonful of chocolate ice-cream. Current need state also appears to modulate the value of an appetitive US from moment to moment; for example, the power of satiation to significantly weaken appetitive conditioning has been demonstrated experimentally (Holland & Rescorla, 1975).

It has been suggested that appetitive stimuli may lack the ability to elicit responses of similar intensity to aversive stimuli (Hermann, Ziegler, Flor & Birbaumer, 2000). Therefore, conditioned responses may be comparatively weaker. This is perhaps unsurprising given the immediate threat and danger associated with aversive stimuli compared to appetitive. Ekman (1992) argued that negative affective states, such as anger, fear and disgust evolved primarily as a survival mechanism to initiate immediate life-preserving behaviour in response to threat. In contrast, positive affective states typically result in a broadening of thoughts and behaviours and may ensure survival in the longer term by promoting seeking of appetitive stimuli and building resources for later use (Fredrickson, Mancuso, Branigan & Tugade, 2000). Whilst it is always pertinent to avoid an electric shock, acquiring ice-cream may not always be such a high priority – for example, if one is ill or has just consumed a large amount of food. Consequently, stimulus value may fluctuate and selecting consistent, high-value USs may pose a greater challenge for appetitive than aversive conditioning research.

Despite these potential challenges, a growing body of evidence supports the feasibility of developing suitable appetitive conditioning paradigms for laboratory

settings. A wide variety of food USs have been used successfully – typically sweet treat foods such as chocolate milk (Meyer et al, 2015), marzipan (Blechert et al., 2016) or milkshake (Burger & Stice, 2014). Savoury snacks, such as pretzels, may also be effective when self-selected by participants (Andreatta & Pauli, 2015; Wardle, Lopez-Gamundi & Flagel, 2018), suggesting that individual tastes and preferences can influence conditioning. In principle, any cue can come to be associated with highly palatable foods and researchers have used a wide variety of CSs, including simple shapes (Meyer, Risbrough, Liang & Boutelle, 2015), novel geometric objects (Blechert et al., 2016), lunch trays (Van Gucht, Vansteenwegen, Van den Bergh & Beckers, 2008), and even emotions paired with an appetitive food US (Bongers & Jansen, 2016).

Furthermore, the interval or time-period between CS presentation and the receipt of the US appears to be a critical factor. It has been demonstrated that conditioning is strongest when the CS predicts the timely delivery of the US, with a short inter-stimulus interval (Costa & Boakes, 2007). However, it has also been proposed that the CS-US interval determines the type of CR (Schachtman & Reilly, 2011). For example, short intervals may elicit focused approach-motivated behaviours and consummatory reactions (i.e., salivation), whilst long intervals may elicit more general seeking behaviours (Akins et al, 1994).

Additionally, Rescorla (1966) proposed that the CS-US contingency was of great importance to the acquisition of a CR. Contingency refers to the relationship between the CS and US, and the probability that the CS will be correlated with the occurrence of the US in some way. As well as this, CS-US belongingness can influence conditionability. Conditioned responses will be more easily acquired for a CS with high belongingness to the US (e.g., pairing an angry face with a scream) than low belongingness (e.g., pairing a landscape image with a scream) (Hamm, Vaitl & Lang, 1989).

Through additional processes, the strength of Pavlovian associations may be modified by altering the degree to which the CS predicts the US. For example, through *extinction*, repeated pairings of the CS in the absence of the US gradually weakens CRs until the CS no longer elicits a response at all (Bouton, 2007) (see Figure 1.7a). Current theories of extinction propose that the representations of the CS-US relationship are preserved, based on phenomena such as spontaneous recovery, where after a significant delay, a previously extinguished CS is reinstated.

As such, extinction is considered a form of inhibitory learning where the CR is suppressed (Bouton, Westbrook, Corcoran & Maren, 2006) rather than erasure of an associative memory (for a review see Dunsmoor, Niv, Daw & Phelps, 2015).

Additionally, *devaluation* (see Figure 1.7b) can occur when a once appetitive CS is somehow made aversive or less desirable, such as by adding an unpleasant taste to a palatable food reward (Holland & Straub, 1979), or if a favoured food suddenly produces illness (Dwyer, 2005).

A final factor deemed important for conditioning strength is the number of CS-US pairings, yet there is little agreement in the literature about the optimum figure. It has generally been assumed in the conditioning literature that learning is a gradual process that develops incrementally (Hull, 1943), and this basic assumption has formed the basis of key theories in the conditioning literature, such as the Rescorla-Wagner model (Rescorla & Wagner, 1972). This model proposes that the strength of learned CS-US associations increases incrementally across consecutive trials, and that an initially weak relationship becomes stronger as more CS-US pairings occur (McSweeney & Murphy, 2014). Consequently, researchers have typically chosen larger numbers of conditioning trials, rather than focusing on the minimum trial number necessary. For example, Franken, Huijding, Nijs, & van Strien (2011) used 100 conditioning trials using sweet and neutral taste stimuli delivered via a gustometer as respective CS+ and CS-. This procedure is time consuming, tiring for participants and the unnatural context is not reflective of a real-life eating episode. It is also impractical to consider taking this paradigm outside of a laboratory setting to investigate these learning processes in the real world.

However, it seems that the large number of stimulus pairings may be largely unnecessary. Assessing basic laboratory animal learning paradigms, Gallistel, Fairhurst and Balsam (2004) found evidence to suggest that learning typically occurred in a more 'all or none' fashion – and that the general assumption of more gradual, incremental learning was likely to be an artefact of group averaging. After reanalysing data at an individual rather than group level from a variety of conditioning paradigms, the authors concluded that subjects typically demonstrate abrupt learning in less than ten trials. This learning often has a rapid, step-like increase in learning curves from an untrained level of responding to a level seen in the well-trained subject, with no further increase in learning evident with additional

trials. Data from conditioned eye blink experiments in rabbits suggested that learning was often apparent after just one trial.

In support of the rapidity of learning, experimental paradigms in people have found successful appetitive conditioning with minimal trial numbers. Viemose et al., (2013) demonstrated appetitive conditioning after 30 pairings of a CS (a novel image) with the US (a spoonful of yoghurt), and conditioned responding persisted 24 hours later suggesting a robust effect. Furthermore, some studies using monetary rewards have demonstrated appetitive conditioning after 20 trials with each CS (Tapia León, Kruse, Stalder, Klucken & Stark, 2018). When participants self-selected a sweet or salty US, only eight pairings of the US and a visual CS were used, yet conditioning was still successful (Andreatta & Pauli, 2018).

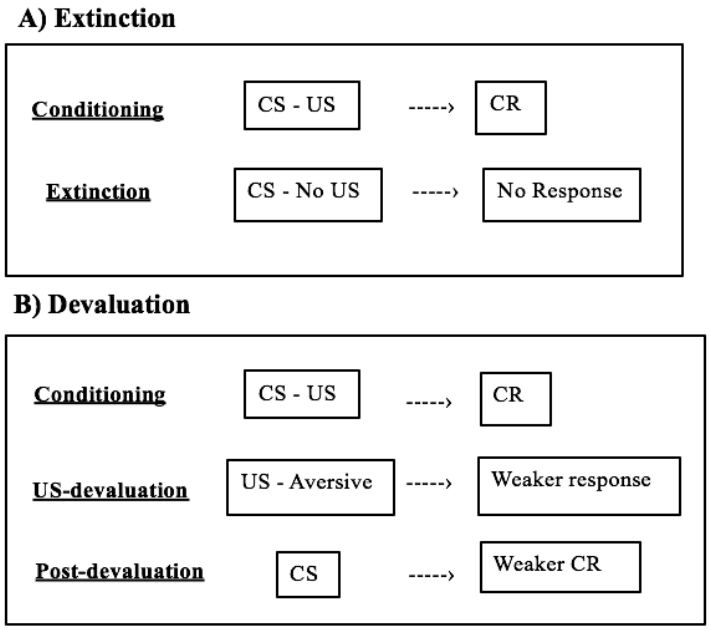


Figure 1.7 A) In Extinction the presentation of a CS in the absence of the US leads to a weakening or absence of the CR. B) The pairing of a US with an unpleasant stimulus leads to devaluation of a CS and weakens the CR.

1.4.4 Single-trial conditioning

Is there evidence to reflect the immediate single trial learning proposed by Gallistel et al. (2004)? Only a small handful of studies have explored the possibility of conditioning occurring in just a single trial, and these have typically been restricted to animals. For example, Essman and Alpern (1964) demonstrated successful aversive conditioning in just a single trial for around 90% of mice tested. Across

several experiments, the authors found that mice would rapidly learn a conditioned avoidance of stepping on a metal plate which previously provided an electric shock. Conditioned taste aversion, has been demonstrated in a single trial in both rodents (Garcia, Kimeldorf & Koelling, 1955; Welzl, D'adamo & Lipp, 2001) and humans (Logue, Ophir & Strauss, 1981). Even the act of observing another chick peck at an aversive substance is enough to cause an avoidance response in 1-day old chicks (Johnston, Burne & Rose, 1998). This may not be too surprising given that most of us will likely recall a time when the taste of a food we have eaten has been closely followed by nausea and immediately elicits disgust. This food may be avoided for years to come, regardless of whether it was responsible for the nausea experienced. Havermans, Salvy & Jansen (2009) found that even exercise was capable of eliciting taste aversions after just a single trial, even in the absence of gastrointestinal discomfort suggesting this to be a powerful and rapid learning phenomenon.

However, most studies demonstrate single-trial learning with aversive stimuli only. This may not be surprising given the immediate and severe, even life-threatening risks associated with many aversive stimuli: failure to avoid a tainted food after an initial experience of illness may have been fatal prior to modern medicine. However, appetitive stimuli are equally relevant for survival and as such, single-trial conditioning should be equally plausible.

In support of this, a limited number of studies have demonstrated successful one trial learning with various appetitive stimuli. Research with male quail provides evidence that single-trial conditioning occurs when the opportunity for sex with a female is paired with a particular context (Hilliard, Nguyan & Domjan, 1997). Additionally, research with both pigeons and baboons demonstrated that one trial learning occurred significantly more than expected when positive rewards were given (Cook & Fagot, 2009). The animals underwent thousands of picture-response association trials and were able to remember a significant number of them even after just one picture-response pairing. The authors operationalised one trial learning as a lack of errors for ten trials or more after they initially learned an association, and these beneficial effects on memory persisted for at least 6 - 8 months after the initial learning procedure took place. The authors concluded that the presence of similar learning patterns in such distantly related animals suggests one trial learning may be present in all organisms.

These mechanisms also appear to be present from very early on in an organism's development. For example, using an odour preference measure, associative memory formation between a neutral odour and a milk CS+ occurred after a single trial in mice that were just 3 days old (Armstrong, DeVito & Cleland, 2006), although the authors did note that learning was significantly strengthened by further learning trials. Nonetheless, this is a clear demonstration of the power of a single experience with a rewarding substance to modify our memories and preferences.

Blechert et al. (2016) designed a particularly powerful Pavlovian conditioning paradigm and presented the first evidence for single-trial appetitive conditioning in humans. This procedure involved edible colour-shape compounds made from marzipan (CS+) and visually similar but inedible objects made from plastic (CS-). The sweet taste of marzipan acted as a US, forming a positive association between the object's unique combination of colour and shape, with its sweet taste. As this was a differential learning paradigm, the colour and shape of the plastic object became associated with a lack of reward. The sight of the CS+ instantly became a highly craved, desirable cue after just a single eating episode. This provides a first insight in to a potent mechanism which may have a profound effect on human eating behaviour.

When interpreting these findings, several methodological features should be considered. Firstly, the authors do not specify the time of day testing took place, which may have impacted on motivation to eat. However, participants were instructed to fast for a minimum of three hours prior to the experiment to induce a state of hunger. This fasting period may have enhanced the effects; satiety has been shown to weaken appetitive conditioning (Holland & Rescorla, 1975).

In addition, participants were exclusively female, comprised of psychology students who were a healthy weight on average. The authors chose to exclude males based on evidence that females may be more susceptible to food cravings than men (Cepeda-Benito, Fernandez & Moreno, 2003). The authors do not report any individual difference measures, such as impulsivity, dietary restraint or trait levels of food cravings so it is impossible to determine whether other characteristics of the sample could account for these findings. Generalizing from student samples is already problematic as they are typically more homogenous than the general

population (Hanel & Vione, 2016). Thus, more research is needed to determine whether certain individuals may be more susceptible to conditioning.

Lastly, BMI was measured at the beginning of the test session, drawing attention to participants' body weight. This sort of cue can act as a prime, perhaps cueing weight loss goals or, alternatively, acting as a disinhibitor (Pacanowski et al., 2015) which may have had an impact on participant responses.

1.4.5 Learning to overeat?

Although there is currently a paucity of research in this area, the relevance of these associative, appetitive conditioning processes to human eating behaviour is clear. The majority of us will have first-hand experience of appetitive conditioning at play: the sight of an initially meaningless cue, the Golden Arches™ from McDonalds, is a clear signal highlighting the availability of fast food. As predicted by the incentive-sensitization theory, such cues may acquire incentive salience through Pavlovian conditioning and elicit wanting (i.e., a strong motivation to obtain the appetitive stimulus) and liking (i.e., the hedonic pleasure associated with an enjoyable experience) (Robinson & Berridge, 2000). Havermans (2013) argues that this mechanism leads to food cue reactivity, where food cues come to elicit physiological preparatory responses (e.g., salivation), approach-motivated behaviours (e.g., food seeking, food purchasing) and subjective responses (e.g., cravings and liking), perhaps leading to unplanned consumption.

Despite difficulties in implementing effective appetitive conditioning paradigms, their power to elicit food cue reactivity and affect eating behaviour has recently been demonstrated in naturalistic settings. For example, when participants' phones were used to provide the instruction to consume chocolate at a specific time of day, they began to experience a stronger desire to eat and greater expectancies regarding receipt of chocolate at that specific time (van den Akker & Jansen, 2017). The authors report that out of a 15-day long experiment, it took just 5 days for this association to reliably form.

Furthermore, in a powerful demonstration of real-world food cue reactivity, participants who ate in a cue-rich environment, as opposed to a neutral environment, experienced greater wanting, felt hungrier, and consumed a higher quantity of food, despite no differences in food liking between the two environments (Joyner, Kim & Gearhardt, 2017). However, the cues that diners were exposed to in this study were highly familiar, common to fast-food restaurants, such as condiment holders,

napkins, menus and plastic trays. Even the classic smell of fried chips was replicated to give as naturalistic an experience as possible. Presumably, the average individual will have prior experience with fast food restaurants. The authors argue that their pattern of results provide clear support for an incentive sensitization model of eating behaviour and through repeated episodes of eating at these establishments, relatively neutral cues have become associated with delicious burgers, milkshakes and fries. Nonetheless, it is worth noting that direct evidence of appetitive conditioning is lacking.

In addition, people will surely have had many other experiences in this same setting: they may have accompanied their children to a birthday party, enjoyed a meal with a new partner, or met with a colleague for a business meeting over coffee. Each individual's specific history with restaurant chains will be different; some will consume fast food frequently, whereas others will only eat at these establishments occasionally. Consequently, further laboratory experiments are needed to better understand the exact constraints underlying appetitive conditioning.

If learning processes and incentive salience are key determinants of eating behaviour, it may follow that they operate differently in individuals prone to overeating. A small number of studies have examined the relationship between appetitive conditioning processes and overweight or obesity in humans – with mixed results. Meyer et al. (2015) found that overweight, but not healthy weight individuals, acquired conditioned responding to a neutral visual cue (a blue circle or red square) paired with chocolate milk delivered via a gustometer. The overweight participants swallowed more in response to the chocolate paired cue (CS+) than the cue paired with water. Healthy weight participants showed no differential responding, suggesting that conditioning was unsuccessful in this group. The authors argue that overweight participants may be more receptive to appetitive conditioning due to their increased sensitivity to the hedonic value of the CS+. However, other findings in this area are very mixed. For example, research has also shown that overweight individuals are poorer than healthy weight individuals at forming CS-US associations with an appetitive CS (Van den Akker, Schyns & Jansen, 2017). Further complicating the story, dieters spent longer in a virtual room paired with chocolate than non-dieters, suggesting a stronger conditioned place preference (Astur et al., 2015). As dieters exert control over their eating and typically avoid eating high energy dense foods such as chocolate, this may run contrary to expectations.

However, it is also possible that by attempting to restrict food intake, the hedonic value and incentive salience of foods such as chocolate could actually increase as dieters feel deprived, thus triggering cravings and consumption.

It seems, therefore, that appetitive conditioning in humans may play some role in the development and maintenance of disordered eating and obesity, although the exact role remains unclear. Clearly, these simple learning mechanisms have been a longstanding aspect of our evolutionary past, with the underlying neural systems having evolved as adaptive mechanisms to ensure survival. Although there are undoubtedly individual differences in sensitivity to the hedonic value of food, this too has likely always been the case. To understand the sudden, recent shift toward overconsumption and overweight, and to move towards effective solutions, it may be important to explore the impact of the environment, as well as human psychology. As homeostatic mechanisms are inadequate for balancing energy intake and expenditure, a rapid change in our environment may have enabled hedonic, incentive and learning mechanisms, adapted to the harsh food environments of our evolutionary past, to create and maintain a positive energy balance

1.5 The obesogenic environment

The mechanisms governing food consumption behaviour evolved in a vastly different food environment from the present day. Humans are thought to have evolved in an environment where food was scarce and its availability varied significantly with the seasons (Foley, 1995). Thus, there would have been huge uncertainty about when, and if, they would secure the next meal. It would therefore be adaptive to eat in anticipation of future deprivation and build up fat reserves for time of famine. Individuals able to eat and store excess fat in this way would be more likely to survive and pass on their genes to their offspring (Ahlstrom et al, 2017).

This harsh reality is now very much in the past; particularly in the last 50 years, our environment has changed dramatically. The rise in energy saving devices and technology such as mobile phones, remote controls, lifts, cars, machinery, just to name a few, has drastically reduced the amount of physical exertion required in our daily lives. However, our diet and energy intake has not been adjusted accordingly, further confirming the inadequacy of energy homeostasis models of appetite control. Rather than restricting energy intake, consumption of energy dense foods has

increased. Even foods that are naturally high in energy are being processed and refined with added fat and sugar (Drewnowski, 2007). People are constantly bombarded with sights and smells of foods and food-related cues and must navigate an environment where food advertisements, logos, vending machines and packaging are ever present and are all designed to entice us to purchase and consume these highly palatable 'junk food' items. The term 'obesogenic environment' has recently been coined to describe this obesity promoting environment.

The availability of fast food outlets, vending machines and convenience stores has increased rapidly and seems to have had a direct influence on overweight and obesity. Positive relationships have been observed between density of fast food outlets and rates of obesity (Li, Harmer, Cardinal, Bosworth & Johnson-Shelton, 2009), and the presence of vending machines was found to be positively correlated with body weight across Canadian schools and colleges (Minaker et al., 2011). These data suggest that proximity and availability of high energy dense foods contributes significantly to poor dietary choices and overeating.

Furthermore, as well as the mere presence of these 'junk foods', marketing companies invest billions in promoting these products and making them more attractive to the consumer. Even while watching a drama or comedy on television we are bombarded by advertisements for junk food items. Evidence suggests that this aggressive food marketing has a direct effect on food intake and body weight; participants exposed to snack food advertisements during eating ate significantly more than when exposed to non-food advertisements (Harris, Bargh & Brownell, 2009). Food intake was unrelated to participants' hunger levels, suggesting that these adverts are tapping in to 'hedonic hunger', and enticing people to eat for pleasure, not need. Perhaps more worryingly, these adverts are most often targeted towards children, and have been heavily implicated in the rise of childhood obesity (Halford, Gillespie, Brown, Pontin & Dovey, 2004). Despite the fact that these marketing strategies may very well be useful for encouraging healthy eating, these adverts are far more commonly advertising unhealthy than unhealthy products (Boyland, Harrold, Kirkham & Halford, 2011). Even though pressure on policy makers has resulted in a very slight decrease in the number of unhealthy food adverts on television, food and drink still has the largest number of adverts in comparison to any other product category (Whalen, Harrold, Child, Halford & Boyland, 2017).

However, it is important to note that, despite the current food environment, not everyone becomes obese. Many people appear able to resist these cues and manage their diet in such a way that they are in a state of energy balance. Therefore, understanding why certain individuals may be more susceptible to these environmental food cues has been a large focus of research, and may lead us towards more efficacious preventative strategies and treatments for obesity.

1.6 Eating with our eyes

It is often said that we eat with our eyes; the visual appearance of food is undoubtedly an important cue in determining eating behaviour. Simply the sight of mouldy or rotten food is enough to turn our stomachs. This automatic aversion to spoiled food is an adaptive mechanism, protecting us from the ingestion of harmful pathogens or toxins (Becker, Flaisch, Rener & Schupp, 2016). However, visual cues also hold a wealth of information about the potential benefits of ingestion; namely, the energy content, nutrient availability and palatability. For example, Toepel, Knebel, Hudry, Le Coutre & Murray (2008) were able to demonstrate using electroencephalography (EEG) that the brain automatically processes and discerns the fat content and palatability of various foods. Additionally, research has shown that humans automatically perceive the colour green as an indicator of low energy density, yet red indicates high energy density – perhaps related to ancestral experience of ripened fruit. Both the nutrient content and palatability of processed foods were judged to be higher when more red hues were present (Foroni, Pergola & Rumiati, 2016).

Vision also appears to be an essential cue, capable of determining not only what is eaten, but how much. Obese diners who ate blindfolded, consumed 24% less food than when they could see the food, and reported feeling just as satisfied by the meal (Barkeling, Linné, Melin & Rooth, 2003). However, this effect appears to be overridden if participants are briefly shown the portion size prior to eating blindfolded (Burger, Fisher & Johnson, 2011). Blindfolded participants ate more from a large portion than a small portion, demonstrating the power of visual cues on eating behaviour. These visual cues also affect satiety and meal satisfaction. Brunstrom et al. (2012) manipulated the amount of soup participants perceived themselves to have eaten by covertly adding or removing soup from the bowls.

Those that saw a larger portion of soup in the bowl, and believed they had consumed it all, felt more satiated.

In the nutrient-poor environments in which humans are thought to have evolved, a heightened sensitivity to visual food cues would have been highly advantageous: humans would have relied heavily on vision to rapidly seek out tasty, nutritious foods. In fact, it has been argued that human colour vision evolved as an adaptation to allow more efficient selection of nutritious fruits and berries from amongst foliage (Regan et al., 2001). However, in today's obesogenic environment, where visual food cues are so heavily abundant, this adaptive mechanism may have become a harmful evolutionary throwback, capable of sabotaging our attempts to control our food intake. The mere sight of food may heighten our desire for it through, what Spence recently termed, 'visual hunger' (Spence, Okajima, Cheok, Petit & Michel, 2015). If these visual cues can influence the brain's reward systems and acquire incentive salience, they may then be the trigger which activates approach motivated behaviours towards highly palatable foods.

1.7 Attentional bias for appetitive stimuli

Human capacity for information processing is limited, therefore, only a small percentage of the environment can be attended to at any one time. The brain must rapidly determine what is of significance in that moment, bringing it to the forefront of attention. Stimuli deemed to be unimportant may be filtered out automatically and fail to reach conscious awareness (Rensink, O'Regan & Clark, 1997). For example, when crossing the street, individuals must be aware of the sight and sound of oncoming vehicles whilst filtering out the chatter of nearby pedestrians, children playing in the nearby park, and the adverts and products prominent in shop window displays. Attending to the vast array of stimuli in our environment in its entirety would render us unable to function, so this ability to selectively attend to the most important stimuli is crucial.

It is theorised that individual differences in how the brain assigns importance to salient stimuli in the environment is related to approach or avoidance motivated behaviour and may contribute to various disorders such as substance abuse and even obesity (Field et al., 2016). Thus, if an item in the environment is particularly attention grabbing, an individual is more likely to either take steps to investigate or

acquire it (approach), or to retreat from it (avoidance), depending on whether it is appetitive or aversive. This enhanced tendency for certain salient items to capture and/hold an individuals' attention is known as an attentional bias. This attentional bias can be measured to assess a participant's level of approach or avoidance motivation for particular stimuli.

It has been widely demonstrated, and corroborated in various meta-analyses and reviews, that a greater attentional bias for threat-related stimuli is associated with the presence of anxiety disorders (McNally, in press). For example, high worriers attend more to threat-related words such as 'cancer' or 'death' than low worriers (Goodwin, Eagleson, Mathews, Yiend & Hirsch, 2017), and individuals high in social anxiety are more likely to show an attentional bias towards human faces (Mogg, Philipott & Bradley, 2004). It has therefore, been argued that these attentional biases are central to the development and maintenance of these disorders.

Attentional biases for appetitive stimuli have also been reliably demonstrated. There is a wide body of evidence supporting the assertion that attentional biases exist for appetitive stimuli such as drugs of abuse and are related to substance use disorders (see Field & Cox, 2008 for a review). In line with the incentive sensitization theory (Robinson & Berridge, 1993), this tendency to allocate greater attentional resources towards appetitive stimuli is thought to reflect the degree of incentive salience assigned to them (Field et al., 2016). Through associative learning, neutral cues become associated with rewarding stimuli and thus become attention grabbing. Once attention is captured by these cues, wanting is triggered and the organism will be drawn to consumption. For example, drug related paraphernalia become associated with the effects of substance use and, via attribution of incentive salience, these drug related cues are then able to capture and hold attention. Theoretically, the next time the individual encounters a drug related cue, it will grab their attention, they will experience cue-elicited cravings and they may find themselves using drugs again, regardless of their intentions. This theory has raised concerns about the potential for anti-drug campaigns to actually have the contrary effect of enhancing cravings rather than dissuading drug users from consumption (Hamed, Zahra, Mehri, Sanju & Azarakhsh, 2010).

There is mounting evidence, using a variety of drug cues and methodologies, which appears to support a role of incentive salience attribution in attentional bias. Using a visual probe task, Field, Mogg, Zettler & Bradley (2004) found that heavy

drinkers maintained attention towards alcohol related pictures longer than light, social drinkers. Furthermore, Kwak, Na, Kim, Kim and Lee (2007) showed using eye-tracking technology that smokers looked significantly longer at smoking related images than non-smokers. Neuroimaging has also shown that ecstasy users have enhanced occipital activation in response to images of ecstasy related cues, thought to reflect enhanced visual processing (Roberts & Garavan, 2013).

There is also evidence that attentional bias may even predict treatment outcomes for alcohol abusers, with those who did not respond to treatment showing an increase in attentional bias for alcohol cues over the treatment period (Cox, Hogan, Christian & Race, 2002). Furthermore, a variety of training techniques have been developed to retrain attentional bias and there is growing evidence for their effectiveness in reducing, not only attentional bias itself, but also in reducing substance use (for a review see Cox, Fadardi, Intriligator & Klinger, 2014), again strengthening the argument for a relationship between attentional bias and approach motivated behaviour.

1.7.1 Food-related attentional bias

Due to the relevance of maintaining energy levels for survival, it makes sense that cognitive processes would also be biased towards the efficient detection and selection of appropriate nutriment. This idea has garnered a breadth of empirical support. For example, in non-clinical samples the sight of food alone has been shown to activate a complex neural network of brain regions implicated in hedonics and food reward (Beaver et al., 2006). Furthermore, Nummenmaa, Hietanen, Calvo & Hyönä (2011) demonstrated a detection advantage for food versus non-food items amongst healthy participants in a visual search task, and high energy dense food cues reliably capture attention more strongly than low energy dense food cues (Doolan et al., 2014). Taken together, this research suggests that attentional circuits may have evolved to selectively attend and orient towards food sources with the aim of maximizing energy intake and nutrient content.

1.7.2 Attention and motivation

It has been reliably demonstrated that motivation and attention interact in the processing of food-related cues. The physiological drive for food can influence how much attention relevant cues attract. Under conditions of hunger, food related words

attract greater attention than when sated (Mogg, Bradley, Hyare & Lee, 1998). Similarly, photographs of palatable foods were found to capture attention more in an emotional blink of attention task (EBA) after a period of fasting (Piech, Pastorino & Zald, 2010). Furthermore, research by di Pelligrino et al. (2011) suggests that attention may fluctuate based on the current value of a food; both selective attention and perceived pleasantness decreased from pre- to post-consumption for a recently eaten food. No such change was evident for a food not recently consumed. Building on this, Davidson, Giesbrecht, Thomas and Kirkham (2018) tracked attentional processing of food-related cues at regular intervals over two hours prior to, and following, a lunch meal eaten to satiety. The researchers observed that attentional capture by food-cues increased as hunger naturally rose prior to a meal, then sharply decreased after food intake – particularly for cues representing the particular food that had been consumed. This seems to indicate that attention serves as a marker of a cue's current incentive salience.

It is important to consider that, the test sessions ran between 10am and 2pm with lunch served at midday; a typical time for lunch to be eaten, which is generally associated with a peak in appetite (Reichenberger et al., 2018). It is likely that effects would be less evident at different times of day when individuals are not experiencing a strong desire to eat. It is unclear whether reward or cognitive processes such as learning would bias attention in this way in the absence of physical hunger.

In addition, although participants in this study were not instructed to fast for a specific time frame prior to the experiment, participants were given explicit instructions not to eat a lunch meal prior to the experiment as a sandwich lunch would be provided. Participants were also asked to choose their preferred sandwich filling for their lunch. These instructions may have acted as a prime, generating expectations and prompting greater food reactivity. Making repeat ratings on appetite scales could also act as a prime, potentially eliciting exaggerated hunger responses, although recent research failed to support this idea (Douglas & Leidy, 2019).

Similar to Blechert et al. (2016), measures of eating related attitudes or individual difference measures were not reported so it is possible sample characteristics are responsible for the observed effects.

1.7.3 Attentional bias and hedonic overeating

Whilst food-related attentional bias appears to be indicative of an inherently normal process, disrupted attentional processing has been heavily implicated in obesity. Evidence seems to suggest that obese individuals show a hyper-responsiveness to food cues (for review see Hendrikse et al., 2015), and that attentional bias may be a central mechanism in hedonic overeating. Compared to healthy weight controls, obese individuals seem to show a greater attentional bias than normal weight controls (Nijs et al., 2010; Castellanos et al., 2009). However, in both studies it seemed that the greatest difference between normal weight and overweight individuals occurred during satiation. Food was equally salient for obese and healthy weight individuals when participants were fasted, but continued to attract attention when sated for overweight participants. This suggests that 'wanting' for food may be enhanced in overweight and obese individuals even in the absence of hunger. However, findings in this area are mixed and it may be premature to conclude that attention for food cues and obesity have a direct relationship (see Doolan, Breslin, Hanna & Gallagher, 2014 for a review).

There is evidence that differences in attentional bias for food play a key role in human eating behaviour. For example, children with greater attentional bias for food cues during advertisements ate more of the advertised snacks (Folkvord, Anschütz, Wiers & Buijzen, 2015). Additionally, stronger attentional bias towards appetizing food cues was positively associated with weight gain at a one-year follow up (Yokum, Stice & Ng, 2011). These biases appear to be present from childhood and may already be exerting an influence over eating behaviour and weight. Werthmann et al., (2015) demonstrated that obese children who displayed rapid orienting toward food cues were less likely to have lost weight at a six-month follow up.

However, it seems that attentional bias can be either helpful or harmful depending on the type of foods which are attended to. Thus, in one study, those who attended more to unhealthy food words were more likely to gain weight over a twelve-week weight loss intervention, while individuals who preferentially attended to healthy food words were more likely to lose weight (Calitri, Pothos, Tapper, Brunstrom & Rogers, 2010).

The tendency to attend to food-related cues appears to be open to modification. Training people to avoid attending to chocolate cues can subsequently

reduce chocolate intake, supporting a link between attention to food and intake and the possibility of modifying behaviour by modifying attention (Werthmann, Field, Roefs, Nederkoorn & Jansen, 2014). These findings appear to extend beyond just chocolate. In another study, participants underwent attentional bias modification training and were trained to attend to either healthy or unhealthy foods (Kakoschke, Kemps, & Tiggemann, 2014). Those trained to attend to healthy foods showed greater attentional bias towards the healthy cues and consumed more healthy snacks compared to unhealthy snacks. The opposite pattern was observed for those trained to attend to unhealthy foods. Again, these findings support the view that what we attend to determines what we eat.

1.8 Mechanisms of Attention

To understand why certain individuals are more easily distracted by visual food cues, it is pertinent to consider exactly how attention is guided. For many years, classical theories of attention have posited that a dichotomy exists, whereby attention is guided by top-down (goal driven) or bottom-up (salience driven) processes (Posner, Snyder & Davidson, 1980).

Bottom-up processes refer to simple visual features of a stimulus that pop out at the individual. For example, a piece of black fluff may automatically and involuntarily capture attention due to its resemblance to a spider, distracting attention away from a current task. Research investigating food-related attentional bias has typically controlled for low-level stimulus features such as brightness and colour by visually matching food and control images (e.g., Nummenmaa et al., 2011), so findings cannot be attributed to such processes.

Conversely, the individual's current goal set is also important: a picture of a snack on a billboard may jump out at a hungry individual on the look-out for food, but remain unnoticed by someone just leaving a restaurant feeling full. As discussed previously, current evidence strongly supports a role of goal-driven attention in food-related attentional bias based on current need state (Mogg et al., 1998). Furthermore, holding food items in working memory also biases attentional capture in a top-down fashion (Higgs, Rutters, Thomas, Naish & Humphreys, 2012), such that those who are already thinking about food are more likely to notice it in the environment. Nonetheless, food cues can automatically capture attention even when their detection

is at odds with the current task (Piech et al., 2010), suggesting that the dual-process model may be incomplete.

1.9 Reward-driven attentional capture

Recent research has identified a third driver of attentional allocation, beyond that explained through either goal set or visual salience – namely, reward-driven or value-driven attentional capture (Munneke, Hoppenbrouwers & Theeuwes, 2015). Proponents argue that previously neutral items paired with reward become salient and attract attention. Through experience, stimuli associated with reward become more likely to enter our awareness and thus guide our behaviour in order to maximise the possibility of gaining reward.

A number of experiments have been conducted to explore this possibility under controlled conditions. In a computer-based learning paradigm, previously neutral stimuli become distracting after being paired with monetary rewards (Anderson, Laurent & Yantis, 2011a). A coloured singleton (e.g., a red circle amongst an array of different coloured circles) which had been paired with a monetary reward consequently captured attention to a greater extent than those of a different colour. Such effects have been replicated with stimulus orientation as well as colour. Thus, Gabor patches oriented in a specific direction that was associated with reward will selectively capture attention, suggesting that any neutral stimulus can acquire this reward-based salience (Laurent, Hall, Anderson & Yantis, 2015).

Even when the researchers alter the task so that colour or orientation no longer signal reward, these features remain attention grabbing. Anderson et al. (2011a) observed that whilst searching for targets based on shape, participants found it impossible to ignore items which appeared in a previously rewarded colour, even though this impaired performance on the new task. The distractors were physically non-salient and participants were explicitly informed that colour was now irrelevant to the task, discounting the possibility that basic visual features or goal-set influenced responding.

Additionally, learning can magnify the salience of already physically salient stimuli: salient stimuli paired with reward captured attention more than physically salient stimuli not paired with reward (Anderson, Laurent & Yantis, 2011b). This effect appears to persist beyond the training phase and only diminishes over several

hundred learning trials, providing evidence that rewards "teach" visual selective attention. In this way, attention is drawn to objects, features and locations which are likely to maximise the possibility of obtaining reward – or avoiding punishment (Chelazzi, Perlato, Santandrea & Della Libera, 2013).

There is also mounting evidence that reward-driven attentional capture is sensitive to the value of the rewarding stimuli. Anderson et al. (2011b) demonstrated that task irrelevant distractors paired with a high value reward (5 cents) subsequently impaired performance to a greater extent than low value (1 cent) reward-paired cues. This effect of value on attention persists even when the learning phase and test phase are separated by up to five days (Della Libera & Chelazzi, 2009). Therefore, it would seem that past experiences are drawn on to infer the potential value of a stimulus at any given time, with reward history being held in memory to guide future decision making and behaviour.

Additionally, Raymond and O'Brien (2009) found evidence that the valence of an association is also important for determining attentional processing and that, under some circumstances, reward may be processed preferentially over punishment. Participants undertook a value-learning task in which different faces were paired with monetary reward or no reward. The influence of the acquired predictive valence of the faces on attention was then assessed in an attentional blink task. Pairs of targets – a neutral abstract geometric pattern (T1) and a face (T2) were presented in rapid succession, 200 ms apart, a condition in which processing of T1 reduces the availability of cognitive resources to attend to T2 (an attentional blink). Participants were required to determine if T1 comprised circles or squares and whether or not T2 had been present in the learning phase. Due to the short lag between targets, the presence of T1 typically impairs detection on T2. However, the researchers found that while this held true for non-reward or loss-associated T2s, the reward associated faces survived the 'attentional blink' effect and were still correctly identified, despite only being presented under conditions of very limited awareness. This suggests that reward-paired stimuli are more likely to reach conscious awareness when attentional resources are limited – they require fewer attentional resources to be selected.

Whilst it has been generally accepted that some form of associative learning is at play here, there has been debate over whether Pavlovian learning, as discussed above, or operant learning can account for these differences. Le Pelley, Pearson, Griffiths and Beesley (2015) argue that, as task irrelevant distractors capture

attention following reward learning, this represents Pavlovian conditioning, which relies on the association between the cue and an appetitive stimulus, rather than operant conditioning where the appetitive stimulus depends on a behavioural response. However, reward delivery was only given on trials where participants gave correct responses, hence, this arguably is not a true case of Pavlovian conditioning and it is difficult to conclude that operant learning plays no role.

Further research by Bucker and Theeuwes (2017) has gone some way to elucidating this confusion. In a test phase, task irrelevant distractors in two different colours were paired with either high or low monetary rewards. Following this, in a non-reward test phase, participants were required to determine the orientation of a line within a shape singleton in the absence of any rewards. During this test the high and low reward-paired distractors appeared in the periphery, and participants were not required to respond to them. The researchers found that performance was poorer on trials where the coloured singletons paired with high reward were present, suggesting that these high value, reward paired cues distracted participants from the task at hand, causing them to miss the target line. They concluded that this effect demonstrates a true case of Pavlovian conditioning, and that this form of associative learning underlies reward driven attentional capture.

Research surrounding value-driven attentional capture and attentional bias related to food or drug use have been conducted entirely separately, by researchers in largely unrelated disciplines. There also appears to be very little discussion between these two large, yet distinct literatures. This may be unsurprising given that there are some key differences between them. For example, research related to reward-driven attentional capture, discussed above, typically makes use of arbitrary neutral or novel stimuli paired with monetary reward, which is a secondary reinforcer and thus has no innate biological value. For example, whilst food is innately reinforcing from birth, the value of money is learned as it is not inherently rewarding. The researchers also maintain tight control over the learning to which participants are subjected. Participants typically undergo thousands of learning trials over a few hours at most. They will also normally be healthy adults, free from any cognitive impairments or reward system dysfunction.

By contrast, participants enrolled in attentional bias research with food or drug related cues are presumed to have a rich history with the rewarding substance. They may be from a clinical sample of addicts or eating disorder patients, or they

may be generally healthy but have a tendency for overconsumption; disruption to reward system functioning may be evident. The researcher has no way of knowing how much learning about the association between the cue and the reward has taken place, or even if it has taken place. Drugs of abuse and palatable foods are also primary reinforcers (biological). Additionally, there is evidence that whilst primary and secondary reinforcers can be equally rewarding, they may be processed via distinct neural mechanisms (Beck, Locke, Savine, Jimura & Braver, 2010).

However, despite these clear differences, crossover between the two lines of enquiry are evident. It seems plausible that the same Pavlovian associative learning mechanism underlying value-driven attentional capture would also underlie an attentional bias for food or drug related stimuli. In fact, in a recent review, Anderson (2016) argues that the two processes are one and the same. He puts forward evidence that five key features of attentional bias are present, regardless of whether the research is concerning arbitrary reward cues or drug related cues. The benefits of greater cross-talk between disciplines could be substantial and together enhance understanding of the basic mechanisms underlying human attention, and provide further insight in to the relevance of attentional bias for conditions related to reward dysfunction, such as overeating. It seems that Pavlovian conditioning between any appetitive or aversive stimuli and a neutral cue should result in an attentional bias. As such this may be a key mechanism underlying food-related attentional bias. A small but growing body of evidence would appear to support this assertion.

1.10 Attentional bias as a measure of conditioning.

Recently, there has been increasing focus on the role of Pavlovian conditioning processes on attention, using a wide variety of primary reinforcers, not just monetary rewards. Again, as with research on substance related attentional bias, the potential clinical significance of this has been explored. Lee, Lim, Lee, Kim and Choi (2009) demonstrated that a simple classical conditioning procedure, where angry faces of a specific gender were paired with an electric shock, was able to produce an attentional bias for the CS+ faces, but not angry faces of the opposite gender, or alternative facial expressions.

In this experiment the researchers utilised a variation on the classic Stroop task, where participants had to rapidly identify the colour of the face, whilst ignoring

the facial expression. Response latencies were significantly longer for the CS+ faces than any of the CS- faces and this effect was more pronounced for individuals with high trait anxiety. The authors argue that this is a demonstration of the mechanism via which attentional bias in anxiety disorders may develop; the brain tracks the level of threat related to certain stimuli and learns to allocate greater attentional resources to those paired with higher levels of threat through experience. However, faces are particularly relevant for humans and it cannot be assumed that these effects would extend to all stimuli. It is entirely plausible that faces present a special case and the evolutionary significance of human facial expression recognition for survival heightens any effects of conditioning.

Nonetheless, other research has demonstrated that even completely neutral CSs (e.g., photographs of pigeons or cars) can become attention grabbing following fear conditioning (Smith, Most, Newsome & Zald, 2006). Using an emotion induced attentional blink task (EBA) they demonstrated that previously neutral stimuli impaired the detection of a target image in a rapid visual stream, after pairing with an aversive noise. This supports the conclusions drawn in the literature surrounding reward-driven attentional capture; the brain constantly tracks the value and relevance of stimuli to an organism and can rapidly update this information, allocating more attentional resources to the most salient items. Perhaps even more importantly, the CSs in the EBA task were task irrelevant and presented under very limited awareness (100 ms). Participants were asked only to detect a rotated target (a rotated landscape image). They were not told to detect the presence of the CS+ and CS- images, or even informed that they would appear in the stream. However, the CS+ was still able to capture attention sufficiently to impair detection of the target, suggesting that conditioned cues can rapidly capture attention even when they are presented outside of awareness, and when their detection is at odds with the individual's current goal state. However, these studies focused on aversive conditioning, so tell us little about the processing of reward paired cues. Could these processes underlie the development and maintenance of an attentional bias for food cues too?

1.10.1 Appetitive conditioning and attention: food as reward

There are a few limited examples of research attempting to explore the modulation of attentional processing by appetitive conditioning. Armel, Pulido, Wixted and Chiba (2009) demonstrated that abstract shapes paired with a food reward elicited more

positive facial expressions of emotion, and participants spent more time looking at the high value images. This experiment suggests that emotion and attention are rapidly modulated by appetitive conditioning. Interestingly, the researchers demonstrated that participants learned to distinguish between cues paired with different USs based on their valence. For example, a picture paired with a delicious US (self-selected ice cream) was rated as more pleasant and looked at for longer than that paired with a less pleasant item (cane sugar) or unpleasant items (baking soda or cornmeal). There was also a linear relationship between valence ratings of the four CSs and implicit pleasantness, measured via subtle movement of facial muscles involved in positive and negative facial expressions. This demonstrates that humans are able to rapidly learn the specific value of initially neutral stimuli and can thus distinguish between a slightly pleasant and very pleasant CS, as well as simply between an appetitive CS and aversive CS.

Whilst this study offers important contributions to the literature, there are several limitations worth noting. Firstly, the study design was very unnatural; participants tasted small amounts of 'food' stuffs such as cane sugar or baking powder while being shown images of abstract shapes. This does not mimic a true eating episode. It is unlikely that participants would ever taste substances such as these in isolation in everyday life. Food is most typically eaten as a compound.

Additionally, attention was measured using video recordings of preferential looking behaviour. This technique, while easy and cheap to record, involves time consuming data analysis by a coder. Therefore, it is subject to error and involves subjective judgements as it relies on the experimenter being able to accurately see when gaze was directed towards each image. It is unclear whether results would hold true when using more robust measures of attention.

As discussed earlier, Blechert et al. (2016) presented a unique paradigm which combined the CS and US to form a novel 3D 'food object'. As well as collecting self-report measures of craving and liking, the researchers also used electroencephalography to examine neural activity in response to images of the CS+ and CS- before and after conditioning. Event related potentials indicated that both early and late neural responses to the CS+ are modulated by single-trial conditioning, suggesting influences to both early and late stages of attention. The authors argue that modulations of the early N1 response may represent an increased vigilance for food cues and suggests an attentional component to conditioning, but, as of yet, these

processes are not well understood. This naturalistic procedure provides a useful framework for modelling the process of experiencing a novel food from first sight, to smell, touch, consumption and the associated hedonic and incentive processes in learning about its properties.

1.11 Learning, motivation and attention

To summarise, learning processes, visual attention and motivation all play a key role in determining behaviour and ensure organisms efficiently procure rewards and avoid punishments, optimising survival. Attentional processing of cues with clinical relevance, such as substance-abuse or food-related attentional bias is often considered in isolation from visual perception research regarding a learned attentional bias for arbitrary reward-paired stimuli, despite evidence suggesting they share a common mechanism (Anderson, 2016).

Due to the brain's limited processing capacity, the most salient stimuli must be selected for further processing, whilst filtering out other irrelevant distractions. Salience can be determined by motivational processes; stimuli with high intrinsic motivational significance can modulate attentional processing. This may be due to the current demands of a particular task (Higgs et al., 2012) or the individual's current need state (e.g., food cues during a state of hunger (Piech et al., 2010)). In addition, motivational significance can be acquired through Pavlovian conditioning processes, guiding attention and motivated behaviour (see Figure 1.8). Learned associations between a US and a neutral CS imbue the CS with motivational significance and determine attentional orienting and selection to that cue, which is then available for further cognitive processing. In the case of an appetitive stimulus such as food, the degree of attention reflects the cue's incentive salience and can also generate motivational responses such as wanting and craving, and hedonic responses related to the cue's affective properties, which can subsequently guide approach-motivated consummatory behaviours (Robinson & Berridge, 2000).

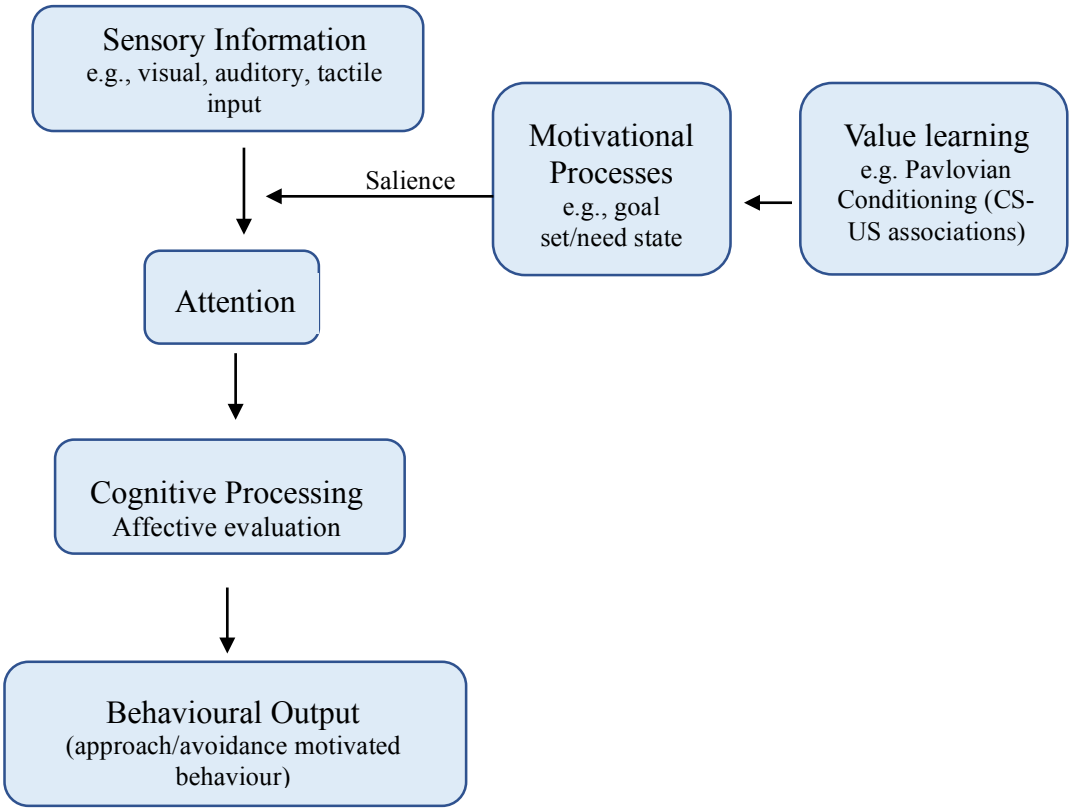


Fig. 1.8 A model of a proposed pathway through which learning, motivation and attention can interact to guide approach and avoidance motivated behaviours. Incoming sensory information is filtered based on salience. Motivationally significant information is highly salient and can selected for further attentional processing. Value learning through Pavlovian conditioning can modify this process by imbuing otherwise neutral cues with motivational salience. As these cues attract attention they become available for higher level processing and guide the organism to making an appropriate behavioural response.

1.12 Outline and hypotheses of the current thesis

Bringing together literature from the fields of learning, visual attention and motivation, the overarching aim of the present thesis was to explore the conditioning mechanisms by which initially neutral stimuli may modulate motivational and attentional processes when associated with a primary appetitive stimulus (in this case food).

Building on the work of Blechert et al. (2016), a core aim was to explore human naturalistic appetitive conditioning by measuring conditioned responses at an explicit level via self-report measures of wanting (e.g., cravings) and liking. A further aim was to use an implicit measure of attention to track changes in the motivational significance of a cue before and after conditioning, based on degree of

attentional capture. It was predicted that using this optimised procedure, which mimics a naturalistic encounter with a new food, conditioning would occur rapidly in as little as a single trial.

Developing greater insight in to how learning, motivation and attentional processes influence approach motivated behaviours in humans may have important implications for our understanding of the physiological, psychological and behavioural mechanisms underlying various disorders related to reward-dysfunction, such as obesity, eating disorders and substance abuse disorders.

The primary hypotheses investigated in the thesis are as follows:

1. A naturalistic appetitive conditioning paradigm will result in the successful acquisition of conditioned responses in a just a single conditioning trial
2. Successful conditioning will be evident on both implicit and explicit measures.
3. Attention to otherwise neutral stimuli will fluctuate relative to their reward history, and may provide an index of incentive salience.

The following goals are central to each chapter:

Chapter 3:

- Replicate Blechert et al.'s (2016) naturalistic single-trial conditioning procedure and investigate modulation of explicit markers of successful conditioning.
- To explore the viability of the EBA task as an implicit measure of incentive salience and value-driven attentional capture.
- To measure modulation of attentional processing by naturalistic conditioning in a single trial.

Chapter 4:

- To isolate and systematically examine the individual contributions of reward, novelty and simple exposure to motivational and attentional processing following a naturalistic single trial conditioning procedure.

Chapter 5:

- To examine how motivational and attentional processing related to a food-paired cue may fluctuate as reward history increases across multiple trials.

Chapter 6:

- To investigate the role of stimulus generalisation in appetitive conditioning at a motivational (self-report) and attentional (EBA) level.

- To explore the influence of stimulus novelty on the acquisition of conditioned responses and the modulation of motivational and attentional processing.

- To explore how stimulus novelty affects the generalisation of conditioned responses to stimuli which share some similarity with a conditioned stimulus.

Chapter 7:

- Firstly, to investigate neural correlates of single-trial appetitive conditioning by examining neural activity during a passive viewing paradigm.

- Secondly, to investigate neural correlates of attentional capture by recently conditioned stimuli in an EBA task.

Chapter Two
General Methodology

Several measures and procedures were consistently employed across studies described throughout this thesis. For clarity, and to prevent repetition, these measures are described in detail below. The primary aim of this chapter is to discuss the theoretical justification for each measure, describe its function, and detail its psychometric properties. Where some variations exist between studies, a general overview of a measure or procedure is provided here, and the exact protocol is described fully in each respective chapter. Additional measures, which are unique to a particular experiment, are not presented here but are described in depth in their respective chapter.

2.1 Screening & inclusion criteria

As this thesis was concerned with the acquisition of appetitive Pavlovian conditioned responses, it was important to exclude participants who were likely to find the unconditioned stimulus unpleasant. In addition, it is unsafe for certain individuals to consume specific substances, particularly as the selected USs (marzipan (chapter 3) and chocolate (chapter 4-7)) are known to contain allergens. Therefore, all participants undertook a basic screening process prior to each experiment. Screening tools used throughout this thesis are described below.

2.1.1 Food liking survey

Prior to participation in each of the experiments conducted, participants completed a screening survey to assess their preferences for the food item (see Appendix A), which would serve as the US in that particular experiment (marzipan in experiments 1 and 2; white chocolate in experiments 3 - 6). As the present thesis was exploring appetitive conditioning, it was important that participants perceived US consumption to be a pleasant rather than unpleasant experience. To hide the importance of the specific US to the experimental aims, participants were required to rate their preferences for nine other food items as well. These were purely filler items which were not used by the researcher. Blechert et al. (2016) reports a similar screening

procedure, which required participants to rate their preference for marzipan on a four-point scale.

A 9-point hedonic scale, originally developed for the US armed forces to assess soldiers' food preferences (Jones, Peryam & Thurstone, 1955), was used throughout this thesis. This scale has nine verbal anchors ranging from '*dislike extremely*' to '*like extremely*', It has been proposed that longer scales with more intervals provide more meaningful levels of relative preference (Peryam & Pilgrim, 1957). However, 9-point hedonic scales are easy to implement, require no training or prior experience, and as well as providing a valid measure of liking, can also adequately predict purchase behaviour (Rosas-Nexticapa, Angulo, & O'Mahony, 2005).

Whilst more sensitive measures exist, such as general labelled magnitude scales (Kalva, Sims, Puentes, Snyder & Bartoshuk, 2014), these may be more complex to implement and are unlikely to provide an advantage when used in the current context. When one is simply measuring the degree of liking for certain foods, there is little convincing evidence that the specific scale used is of much importance (Wichchukit & O'Mahony, 2015).

In each of the six experiments presented in this thesis, participants were required to provide a score of six or above for the US food in order to be invited to take part in the full experiment. As the thesis was exploring what was predicted to be general adaptive mechanisms, a rather conservative score of six was chosen as the minimum for inclusion. By choosing only those that scored very highly, there was a risk that participant selection would be limited to those particularly responsive to sweet tastes and would fail to model general processes underlying food-related attentional bias in non-clinical populations. However, exclusion of those that were likely to dislike the product was also important.

2.1.2 Medical history questionnaire

Prior to experimentation all participants completed a short medical history questionnaire asking participants to list any medical conditions, use of medications, allergies or intolerances which might have impacted on the study aims or participant safety. Individuals with any allergies, intolerances or illness (i.e., diabetes) affected by the ingestion of any of the food used in the experiments were excluded from participation on health and safety grounds. Participants taking certain medications

known to affect vision, attention, appetite and taste were also excluded due to the possible impact of the experimental aims. These medications included drugs such as chemotherapy agents, anti-depressants thyroid medication, mood stabilisers, corticosteroids or antibiotics (see Pilgrim et al., 2015; Douglass & Heckman, 2010; Jaanus, 1992).

2.1.3 Other demographics

Participants were also asked to record their age and anyone falling outside of the specified age range (18 – 40 years) was excluded. This thesis was concerned with the study of learning, motivational and attentional processes in healthy adults. Evidence suggests visual acuity significantly declines beyond the age of 40 and can impair performance in attentional blink paradigms (Georgiou-Karistianis et al., 2007), a version of which was used throughout this thesis. Participants were also asked to confirm verbally that they had normal, or corrected to normal, vision.

2.2 Pavlovian conditioning: Single-trial naturalistic conditioning procedure

A recent study conducted by Blechert et al. (2016) demonstrated the power of a novel naturalistic conditioning procedure to modulate attentional and motivational processes in just a single trial. Due to the apparent strength of this procedure and its potential utility for modelling learning during naturalistic food seeking and consumption (see Chapter 1 for further discussion), this paradigm was replicated and adapted throughout this thesis. The specific protocol for each experiment will be presented in the respective chapters, but a general overview of this conditioning paradigm is presented below.

The conditioning paradigm in the present thesis involved the presentation of 3D objects made from an edible substance (CS+) and visually similar objects made from plastic (CS-) (see Figure 2.1). Each object type was made in a particular colour-shape combination with approximate dimensions, 3cm × 3cm × 2cm. As the CS and US formed a compound it was important to consider edibility when selecting appropriate colours. Colour provides rich information about a food such as its safety, freshness and flavour (Spence et al, 2010). For example, certain colours, such as blue, may be avoided due to associations with poison (Kanig, 1955), whereas reds, oranges and yellows are often considered more appetising (Birren, 1963). The chosen shapes were generally novel 3D shapes, to have no prior associations with other

stimuli. However, familiar shapes were deliberately selected in Chapter 6 to explore the influence of stimulus familiarity and novelty on conditioning. The colour-shape combinations paired with an appetitive and neutral outcome were counterbalanced across participants.

Pavlovian conditioning paradigms involve a pre-conditioning phase, where responses to the CS stimuli are measured prior to any associations with the US, followed by an acquisition phase, where participants learn about the CS-US relationship, and finally a post-conditioning phase where the CSs are presented again and conditioned responses are measured. CS+ objects were custom made to order by specialist confectioners from marzipan (Love Marzipan; Chapter 3) and white chocolate (Chocolate on Chocolate Ltd; Chapters 4 - 7). During an acquisition phase, participants were presented with the CS+ and asked to look at, handle, smell and eat the object. They were also presented with the CS- and asked to look at, handle and smell the object. Through consumption/handling the CS (sight of the novel 3D colour-shape combination) became associated with the US (food/plastic).

Participants were also given 100 mm unipolar VAS scales and asked to rate their degree of liking for each sensory attribute from 'not at all' to 'like extremely' for each CS type. Three scales were completed for the CS- (visual appearance, texture and smell) and four scales were completed for the CS+ (visual appearance, texture, smell and taste). This was done to ensure participants focused on the CS-US contingencies and paid attention during conditioning. Additionally, the taste ratings provided useful descriptive information of the degree to which the US was enjoyed.

As a core aim of this thesis was to investigate the potential for single-trial conditioning, participants underwent only one learning trial in all but one experiment (Experiment 4, Chapter 5) which involved four conditioning trials.

Figure 2.1 Examples of CS+ and CS- stimuli used throughout this thesis.

2.3 Subjective measures

2.3.1 Computer-based image ratings task; Visual analogue scales (VAS)

Visual analogue scales (VAS) can be applied to a wide range of experiences. In particular, VAS have become commonplace for investigating affective experience. Whilst numerous methods exist for measuring the strength of valence associated with a stimulus, VAS have a number of distinct advantages. In particular, they allow a continuous measurement from 0-100, providing rich quantitative data, as opposed to alternatives such as Likert scales which offer very limited response options (Lishner, Cooter & Zald, 2008).

In each valence task, participants rated a random sample of images of each category type used during the emotional blink of attention tasks (CS+, CS-, neutral, dessert, etc.). Each image was rated for valence on a 100 mm VAS, anchored with the terms 'Extremely pleasant' and 'Extremely unpleasant'. Participants were instructed to use the middle of the scale to represent neutral valence or 'no strong feelings', whereas the two end points were to represent the most or least pleasant stimuli imaginable.

In Chapter 3, the valence task scores were used to provide pre- to post-conditioning valence scores for the CS+ and CS- to assess evaluative conditioning processes, as well as to provide a measure of valence for each category of images present in the EBA. However, as the CS+ and CS- were photographed against various household backgrounds in the EBA task, it seemed participants were rating based on the image overall and thus was not giving an accurate score for the valence of the CS stimuli specifically. Consequently, in later experiments an additional measure was included to explore changes in liking of the CS (described below) and the VAS task was used purely for consistency to assess image valence on the EBA task. This task was also adapted in Experiment 3 (Chapter 4) to assess image familiarity.

2.3.2 Food Craving Questionnaire – State Version (FCQ-S; Cepeda-Benito, Gleaves, Williams & Erath, 2000)

The FCQ-S is a fifteen-item scale developed by Cepeda-Benito et al. (2000) to assess food cravings as a psychological state. Participants were asked to indicate how strongly they agreed with each statement "right now, at this very

moment" using a 5-point Likert scale that ranged from 1 (strongly agree) to 5 (strongly disagree). In line with Blechert et al. (2016) participants were asked specifically about their cravings for sweet foods. For example, "My desire to eat sweet foods seems overpowering".

The FCQ-S has been well validated and has been found to measure five factors; 'An Intense Desire to Eat', 'Anticipation of Positive Reinforcement', 'Anticipation of Relief From Negative States and Feelings', 'Preoccupation With Food and Lack of Control Over Eating' and 'Feelings of Hunger. Moreno et al. (2008) demonstrated that the full scale had an alpha-level of .83, and each factor had an alpha of .92, .81, .85, .75 and .69 respectively. The total scale has good construct validity and has been shown to be sensitive to food deprivation manipulations (Cepeda-Benito et al., 2000).

In a replication of Blechert et al. (2016) the FCQ-S was used as a measure of conditioned cravings in Experiment 1 and 2 (Chapter 3), to tap in to wanting as well as liking of the CS+. However, the experimenter observed that this measure did not seem to truly capture cravings elicited by the CS+, but instead measured general cravings for sweet foods at each time-point. As participants were asked to fast for several hours prior to arrival, cravings for sweet foods should generally be quite high prior to conditioning, and little change would be expected after such a small eating episode. However, the question wording made it difficult to assess whether the CS+ specifically was capable of eliciting cravings. As our primary aims were concerned with determining whether a CS+ could be imbued with motivational salience and wanting in a single trial, a scale more appropriate for this purpose was selected for Experiments 3-6 (Chapter 4-7).

2.3.3 Conditioned Responses: Craving, US-Expectancy and Liking

In light of limitations discussed above, self-report measures of conditioning, adapted from Papachristou et al. (2013) were applied in Experiments 3-6 (Chapters 4-7). Participants were shown a photograph of the CS+ and CS- both before and after conditioning and asked to rate cravings, US-expectancies and liking using 100mm VAS (See Table 2.1 for question wording and anchor points). The order of question type and stimulus type were randomised.

Table 2.1 Wording of questions and anchor points used on visual analogue scales presented during a self-report task where participants were required to rate the degree of liking, cravings and expectancies elicited by a CS+ and CS-.

Measure	***Question***	***Anchors***
Cravings	*"When presented with this object, how strong is your craving for chocolate right now?"*	*"No craving at all" to "Extremely strong craving".*
US-Expectancy	*"When presented with this object, how strongly do you now expect to be invited to eat chocolate?"*	*"Certainly not" to "Certainly".*
Liking	*"How pleasant do you find this object?"*	*"Extremely unpleasant" to "Extremely pleasant"*

2.3.4 Self-reported appetite: visual analogue scales (VAS)

The experience of appetite, satiety and hunger are difficult to define and many techniques designed to measure both the physiological and psychological components of these constructs exist. Due to their ease of use and availability, visual analogue scales (VAS) for self-reported appetite have become the most widely used approach (Livingstone et al., 2000). There are a wide variety of scales designed to measure various aspects of appetite, such as the subjective experience of hunger or fullness, somatic sensations including stomach emptiness, the desire to consume certain foods, or the amount of food an individual wishes to consume (Blundell et al., 2010) – all of which have considerable limitations.

When completing VAS scales, participants make ratings by placing a vertical mark anywhere on a 100 mm line between two extreme anchor points. Ratings are quantified by measuring the distance from the left anchor to the point at which participants mark the line: this value reflects the strength of feeling or sensation associated with a particular statement (Hofmans & Theuns, 2008).

Appetite VAS scales exhibit high reliability and validity (Stubbs et al., 2000); they are sensitive to a number of experimental manipulations, and can predict both the type and amount of foods eaten (De Graaf, 1993). They also have high test-retest

reliability, and are thus appropriate for use in within-subjects designs and can be repeated at multiple time points (Stubbs et al., 2000).

Although there are typically strong correlations between different appetite scales, some researchers have highlighted differences in sensitivity and reliability of different measures (Merrill, Kramer, Cardello & Schutz, 2002); therefore, the use of multiple appetite scales is encouraged. Consequently, in each of the experiments reported here, participants' motivation to eat was measured using four well-validated appetite scales, both before and after conditioning took place (see Table 2.2).

Table 2.2 VAS scales for measuring self-reported appetite used in experiments 1-6.

Scale	**Question**	**Anchors**
Hunger	How hungry do you feel?	Not at all - Extremely
Desire to Eat	How strong is your desire to eat?	Not strong at all - Extremely strong
Fullness	How full are you?	Not at all - Extremely
Prospective Consumption	How much food do you think you could eat?	None at all - An extremely large amount

2.3.5 Contingency awareness

Although some evidence suggests conditioning can occur without awareness in certain circumstances, there is a consensus that conscious knowledge of the contingency between the CS and US is necessary for learning to take place (Lovibond & Shanks, 2002). Consequently, at the end of each experiment participants were asked to recall the colour and shape associated with the CS+ and CS- and were classed as contingency aware or unaware dependent on whether their responses were accurate. All participants were classed as contingency aware throughout this thesis.

2.3.6 Questionnaires

Although no direct predictions were made about the influence of individual differences on conditioning or attention, several measures were taken throughout this thesis to asses trait based differences in eating attitudes or reward sensitivity and identify any outliers. Decisions about the scales to include were made on the basis of prior work and theoretical justifications. Each of the four scales are discussed below.

2.3.6.1 Dutch Eating Behaviour Questionnaire (DEBQ; Van Strien, Frijters, Bergers & Defares, 1986)

The widely used Dutch Eating Behaviour Questionnaire is designed to measure distinct eating attitudes and behaviour, and has been widely used in both clinical and non-clinical, healthy samples (e.g., Baños et al, 2014). It has been translated and validated for use in various languages across the world, including English (Wardle, 1987), Turkish (Bozan, Bas & Hulya Asci, 2011) and Spanish (Cebolla, Barrada, van Strien, Oliver & Baños, 2014), and typically demonstrates high reliability and validity. The DEBQ measures three distinct factors related to obesity and overeating: restraint, emotional eating and external eating. This 33-item scale has a 5-point response format ranging from 1 = Never to 5 = Very often.

The term restraint has become synonymous with dieting; it is an attempt to exert control over, and self-regulate one's food intake. This self-regulation seems to be important for controlling body weight, particularly in today's society where food is so abundant (Johnson, Pratt & Wardle, 2012). The ten-item restraint subscale of the DEBQ assesses an individual's intention to limit their food intake, as well as their actual restraint behaviour (Larsen, Van Strien, Eisinga, Herman & Engels, 2007) (e.g. "Do you avoid fattening foods?").

Emotional eating refers to a tendency to eat in response to both positive and negative emotions (Bongers & Jansen, 2016). It is thought that emotional eaters are more vulnerable to overeating due to a reduced ability to regulate emotions effectively (Evers, Stok & de Ridder, 2010). The emotional eating subscale has 13 items (e.g. "Do you have a desire to eat when you are frightened?").

Finally, external eating refers to an individual's sensitivity to external cues in their environment (Herman & Polivy, 2008). For example, high scorers on the external eating sub-scale of the DEBQ are more likely to overeat in response to

watching food advertisements than low scorers (Van Strien, Herman & Anschutz, 2012). The external eating subscale has 10 items (e.g. "If you walk past a bakery, do you have the desire to buy something delicious?"). Mean scores are obtained by adding the scores of the items of the each subscale and dividing by the number of items endorsed on the scale. Only one item is reverse scored, from the external eating subscale, ("Can you resist eating delicious foods?"). Higher scores reflect higher levels of eating attitudes. For example, higher scores on the restraint subscale indicate greater levels of dietary restraint.

Confirmatory factor analysis has demonstrated high stability of the DEBQ (Wardle, 1987). When measured in a non-clinical sample ranging from normal weight to obese, each subscale had high internal consistency: coefficient alphas for the restraint sub-scale ranged from .92 - .94; from .96 -.97 for the emotional eating sub-scale, and, from .79 - .84 for the external eating subscale, (Bohrer, Forbush & Hunt, 2015). However, it is important to note that studies assessing the concurrent validity have found mixed results (Domoff, Meers, Koball & Musher-Eizenman, 2014), as these sub-scales are not always consistent with other well-established measures of the same construct. Inconsistencies are most likely due to methodological differences between studies and difficulties measuring these complex, variable traits in a laboratory setting. Despite these limitations, recent evidence indicates good ecological validity for the DEBQ overall, suggesting that responses truly reflect participants' real world experiences (Mason et al., 2017).

2.3.6.2 Food Neophobia Scale (FNS; Pliner & Hobden, 1992)

Food neophobia refers to a relatively stable trait whereby an individual is reluctant to eat foods which are unfamiliar (Lähteenmäki & Arvola, 2001). This phenomenon is hypothesised to be an adaptive trait which protects individuals from ingesting potentially dangerous food stuffs (Reilly, 2018). As well as an unwillingness to try new foods, neophobics also enjoy new foods to a lesser extent than neophilics, providing lower liking ratings, and harbouring more negative expectancies about novel food (Raudenbush & Frank, 1999).

Although there is currently no evidence to suggest food neophobia and appetitive conditioning are related, it is possible that, due to the novel visual appearance of our CS+, levels of food neophobia could impact on both willingness to

taste, and the perceived enjoyment and reward value of the CS+. Food neophobia was thus measured in each experiment to provide descriptive statistics about the sample and to identify any extreme scores.

The simplest and most widely used measure of food neophobia suitable for adults is the food neophobia scale (Pliner & Hobden, 1992). This 10-item scale requires respondents to make responses on a 7-point scale from 1 (strongly disagree) to 7 (strongly agree). Half of the items are reverse scored. On average, scores for adults are approximately 35, but scores can range from 10-70). Low scores indicate higher levels of neophobia, whereas neophilia is associated with higher scores.

The FNS has high internal consistency; Chronbach's alpha = .088 (Damsbo-Svendsena, BomFrøst & Olsen, 2017). Pliner, Lahteenmaki and Tuorila (1998) demonstrated an association between FNS scores and both willingness to try unfamiliar foods, and the degree of liking for those foods once tasted. This suggests the FNS captures both the expectations and actual sensory experiences of neophobics when exposed to an unfamiliar food. The original 10-item scale was selected for use throughout this thesis as it is the most widely used and well validated version of food neophobia.

2.3.6.3 8-Item Brief Sensation Seeking Scale (BSSS-8; Hoyle et al, 2002)

As early as 1969, Zuckerman suggested the concept of sensation seeking; a personality trait he believed could moderate an individual's propensity to seek out hedonic experiences, and to take risks in order to achieve those experiences. He argued that while humans are motivated to achieve an optimum level of arousal, certain individuals might rapidly habituate to arousing stimuli and thus need to seek out increasingly arousing experiences in order to reach this state. Sensation seekers therefore, pursue novelty, variety and intense stimulation in a variety of ways such as drug use, risky behaviours and overconsumption of palatable foods (Zuckerman, 1971).

Zuckerman's Sensation Seeking Scale Form V (SSS-V; Zuckerman et al, 1979, revised by Zuckerman, 1994) was originally developed to measure this trait and has been heavily utilised and well validated. However, it is lengthy and has been criticised for having a forced-choice response format, as well as using outdated colloquialisms (Saletti, Chang, Pérez-Aranibar & Campos, 2017). Therefore, Hoyle et al. (2002) developed the 8-item brief sensation seeking scale (BSSS) with the aim

of addressing these various shortcomings whilst maintaining the basic structure of Zuckerman's SSS-V. The final version of the 8-item BSSS has two questions addressing each of the same four factors identified by Zuckerman in the SSS-V; experience seeking, boredom susceptibility, thrill and adventure seeking and disinhibition. Chronbach's alpha for the full 8-item BSSS was 0.76, demonstrating acceptable internal consistency. Responses are made on 5-item Likert scales with response options from 'strongly disagree' to 'strongly agree'.

The scale has been translated from English for use in various languages including Chinese (Chen et al., 2013), Hungarian (Mayer, Lukács & Pauler, 2012), Spanish (Stephenson, Velez, Chalela, Ramirez & Hoyle, 2007), Turkish (Celik & Turan, 2016) and Italian (Primi, Narducci, Benedetti, Donati & Chiesi, 2011).

As Zuckerman's theories predict, the 8-item BSSS positively correlates with risky behaviour such as alcohol and cigarette use (Stephenson et al, 2007), and binge eating (Laghi, Pompili, Baumgartner & Baiocco, 2015). It is even predictive of preferences for types of holiday, with sensation seekers preferring adventurous getaways (Eachus, 2004). Therefore, it appears to be a fast, valid and reliable measure of sensation seeking which is easy to implement

2.3.6.4 BIS/BAS Scale (Carver & White, 1994)

Gray (1981) proposed that two opposing systems are responsible for approach motivation and avoidance motivation, and that individual differences in the sensitivity of these systems to reward or punishment related cues in the environment can influence behaviour. In his Reinforcement Sensitivity Theory, Gray (1982) argued that the Behavioural Inhibition System (BIS) regulates the aversive motivation system, whereas the Behavioural Activation System (BAS) regulates the appetitive motivation system.

One of the most well-known and widely used measures of the BIS and BAS is the BIS/BAS scale; a 24-item self-report questionnaire developed by Carver and White (1994). Participants read a series of 24 statements and are asked to indicate their level of agreement with each statement. Responses are made on a 4-point Likert scale with the following response options; 1 (very true for me), 2 (somewhat true for me), 3 (somewhat false for me), and 4 (very false for me).

Factor analysis identified four subscales, one of which corresponds to the BIS (7 items) and three which correspond to the BAS; BAS Drive (4 items), BAS Reward

Responsiveness (4 items) and BAS Fun Seeking (4 items). There are also four additional filler questions (e.g., 'How I dress is important to me'). BAS Drive measures an individual's motivation to follow their goals (e.g., 'I go out of my way to get things I want'), and BAS Reward Responsiveness refers to an individual's ability to experience pleasure in response to the presence or expectation of reward-related stimuli (e.g., 'When I see an opportunity for something I like, I get excited right away'). BAS Fun Seeking measures motivation to approach novel rewards or events spontaneously (e.g., 'I'm always willing to try something new if I think it will be fun'). BAS reward responsiveness seem to reflect an individuals' reward sensitivity, whereas fun seeking may correspond better to impulsivity (Braddock et al., 2011).

The three BAS subscales loaded strongly on to a second-order factor and the BIS subscale loaded on to another factor. Chronbach's alpha for each subscale ranged from .66 to .76, indicating acceptable internal consistency. Test re-test correlations on scores collected 8 weeks apart ranged from .59 to .69, demonstrating good reliability.

There has been much debate about whether there is a true four factor structure, or whether a two factor structure provides a better fit. Although some argue in favour of a unidimensional model of BAS (Maack & Ebesutani, 2018), most researchers seem to agree that the three BAS subscales should be treated as separate constructs despite intercorrelations between them (Ross, Millis, Bonebright & Bailley, 2002; Voigt et al, 2009).

Although the majority of research has failed to find an association between reward sensitivity and appetitive conditioning (Papachristou, Nederkoorn, Beunen & Jansen, 2013), theoretically, it has been proposed that reward sensitivity could facilitate appetitive conditioning (Zinbarg & Mohlman, 1998). Consequently, BAS-RR scores were measured in Chapters 4-7 to assess trait-based differences between participants and highlight any outliers.

2.4 Measures of attention

Humans are constantly bombarded with sensory information; sights, sounds, smells, textures and tastes all compete for processing. Due to the brain's limited processing capacity, it is important that attention can be focused on important stimuli whilst

filtering out irrelevant stimuli. As early as 1890, William James summarised attention simply as the ‘‘withdrawal from some things in order to deal effectively with others.’’ Selective attentional processes determine which stimuli ‘win’ the competition for cognitive resources and enter conscious awareness (Desimone & Duncan, 1995). Attention may be prioritised in a voluntary fashion, based on current goals (e.g. looking for a friend in a crowded bar) and involuntarily due to physical salience (e.g. turning when you hear a friend shout you) or motivational salience (e.g. spotting a McDonalds whilst looking for a taxi) (Anderson et al., 2011a; Most et al, 2007). Anderson and colleagues argue that whilst some motivationally relevant stimuli are inherently salient, others are imbued with salience due to associations with reward (Anderson et al., 2011a; Anderson, 2013) (see Chapter 1 for further discussion).

The limitations of attentional processing can result in two involuntary processes; inattentional blindness and attentional capture, both of which reflect failures to detect stimuli that are clearly visible. Inattentional blindness, refers to a phenomenon where unexpected stimuli are missed when attention is engaged in another task (Mack & Rock, 1998). In a classic experiment by Simons and Chabris (1999), the experimenters demonstrated that participants who were asked to count the number of passes made between players during a game of basketball subsequently missed a man walking in plain sight wearing a gorilla suit. Spatially, participants were attending to the area where the gorilla appeared, but due to limitations on processing capacity, the gorilla went unnoticed when participants were engaged in another task.

Conversely, attentional capture, which is the focus of the present thesis, can occur when salient but task-irrelevant stimuli attract attention unintentionally (Theeuwes, 1992; Forster & Lavie, 2008). This can occur at an explicit level, where the unattended stimuli reach conscious awareness, or implicitly, where attentional capture can interfere with performance on a set task, regardless of whether this is consciously perceived. A measure of attentional capture, the attentional blink, involves the failure to detect an expected stimulus when it is presented shortly after another stimulus within a Rapid serial visual presentation (RSVP) stream (Beanland & Pammer, 2011). The present thesis is concerned with implicit attentional capture measured via modified versions of an attentional blink paradigm, which is discussed below.

2.4.1 Attentional blink

Broadbent and Broadbent (1987) were the first to demonstrate limits of attentional processing via the observation that one target presented in a RSVP task is relatively easy to detect, but the identification of a first target impairs detection of a second target if presented soon after. Raymond, Shapiro and Arnell coined the term 'attentional blink'(AB) in 1992 to describe this phenomenon. When the first target (T1) and the second target (T2) are presented in close temporal proximity (approximately 200-500 ms apart), detection of T1 prevents the accurate detection of T2; yet T2 can still be detected if participants are instructed to ignore T1 (Shapiro, Raymond & Arnell, 1997).

A number of theories have been proposed to account for the AB effect; however, a review by Dux and Marois (2009) concluded that no one theory can adequately account for all existing findings. The authors suggested that the AB involves the enhancement of the representation of T1, which, due to limitations on human attentional processing, renders attentional resources unavailable for a brief window, thus inhibiting processing of further stimuli. It is thought that stimuli compete for attentional priority; T1 typically 'wins' the competition in standard dual-target AB tasks due to the participant's goal set. However, factors that moderate the salience of targets and accompanying, task-irrelevant stimuli within an RSVP stream have been shown to affect the AB, as higher priority is assigned to the more salient items.

2.4.2 Emotional Blink of Attention (EBA)

In recent years, attention has turned towards the influence of affective stimuli on the attentional blink. Most, Chun, Widders and Zald (2005) were first to demonstrate that affective stimuli (e.g. images of graphic violence) can cause a blink even when they are task irrelevant and do not require a response. In single-target paradigms, a critical distractor appears ahead of a specific target. Affect-related AB is most prominent when an emotionally salient distractor image appears 200 ms before a target (lag-2), but becomes progressively weaker as the lag increases, until lag-8 where a blink effect is typically absent (McHugo, Olatunji & Zald, 2013). It has since been shown that the EBA is sensitive to stimuli of both positive and negative valence (Most, Smith, Cooter, Levy & Zald, 2007). Erotic images, as well as violent

images, were found to be effective distractors when presented 200 ms, but not 800 ms before a target.

Smith, Most, Newsome & Zald (2006) also demonstrated that the EBA is sensitive to changes in the affective properties of stimuli. Through Pavlovian conditioning, neutral distractor images were paired with an aversive or neutral outcome. In a subsequent EBA task, distractors with neutral valence were easily ignored; however, stimuli which had recently acquired negative valence, after pairings with electric shocks, involuntarily captured attention. This was the first study to highlight the possibility that the EBA task could potentially track variations in the valence of stimuli over time. Piech, et al. (2010) found further evidence to support this assertion in their observation that the blink induced by food distractor images was greater when participants were fasted compared to when they were sated. This suggests that the value of a stimulus can vary according to current need state.

Building on this, Davidson, Giesbrecht, Thomas & Kirkham (2018) demonstrated the usefulness of the EBA for tracking changes in incentive motivation for appetitive stimuli over time. The researchers measured changes in selective attention to food cues in response to natural variations in hunger and satiety. Participants repeated the EBA task at regular intervals before and after a lunch time meal. When images of food, including the specific lunch items (sandwiches) served as distractors at lag-2, response accuracy of target detection gradually decreased in line with rising hunger levels, reaching their lowest levels just before lunch was served. Thus, motivationally relevant stimuli more effectively induced an attentional blink as motivation increased. Following lunch, accuracy significantly improved again for these sandwich cues, and particularly for images of the specific sandwich type that had been eaten to satiety. Dessert images, which retain high intrinsic hedonic value, remained highly distracting even when participants were sated. The authors suggest that this pattern reflects an attentional component of eating motivation and of sensory specific satiety, and demonstrates the utility of the EBA task for implicitly measuring rapid fluctuations in the motivational value of specific stimuli.

Consequently, the EBA task seems to be a useful tool with which to address our research aims. The present research would suggest that the EBA can provide an index of the incentive salience of a stimulus and should be sensitive to changes in stimulus salience imbued by reward learning.

2.4.3 The present thesis: EBA paradigm

Each experiment in this thesis utilised modified versions of the EBA task described by Piech et al. (2010) and Davidson et al. (2018) to track changes in the motivational salience of an item based on its learned value. The EBA task was completed both before and after the conditioning phase, in a pre/post design. As task difficulty is high, participants' initial performance is typically poor. Therefore, participants completed an initial practice session – which was excluded from analysis, enabling participants to become competent at the task, and to minimize practice effects from pre- to post-conditioning trials (Davidson, 2015), which are typically large with the EBA task (Piech et al., 2010). After a short break, participants then completed the EBA task again, to collect meaningful pre-conditioning measures of attention, followed by a post-conditioning session immediately after.

On each trial (see Figure 2.2 for a schematic representation), participants were required to detect a target image within rapidly presented streams of 17 images. Each image was presented for 100 ms, with no interval between successive images. Fifteen of these images were non-critical, neutral distractor images consisting of natural landscapes, such as trees and mountains, or buildings. There was also a critical distractor, and a target image. The target was always a rotated landscape, whilst critical distractor images consisted of neutral images, images of the CS+, images of the CS-, visually similar unconditioned stimuli and dessert images, depending on the aims of each specific experiment.

Immediately after each stream, participants were prompted to indicate using the keyboard, first, the presence of a rotated image ('did you see a rotated landscape image? Y/N') and then its direction ('which way was the image turned? Left/Right'). Participants were required to correctly identify both the presence and direction of a target image to be classed as a correct response. The critical distractor was to be ignored and participants were not required to make a response: participants were explicitly instructed to identify the rotated target and ignore task-unrelated stimuli.

In this version of the EBA, the critical distractor was always presented 200 ms before the target, as this has been reliably shown to be an optimum time lag to generate a robust attentional blink. Previous research has measured attentional capture when the distractor image appears at longer lags; i.e., 3-8 places before the

target (lag 3-8). As performance recovers rapidly between lags 3 to 8 we chose not to include trials at various lags. This restriction minimized participant fatigue, as fewer trials were needed.

Performance on the EBA task was determined by calculating the percentage of correct responses (correct identification of target presence and direction) per distractor category. As salient distractors should automatically capture attention, thus impairing detection of the target, low accuracy was considered to reflect the 'blindness' caused by an emotional attentional blink. Performance before and after conditioning for each distractor type was compared to assess changes in degree of attentional capture by reward-paired cues.

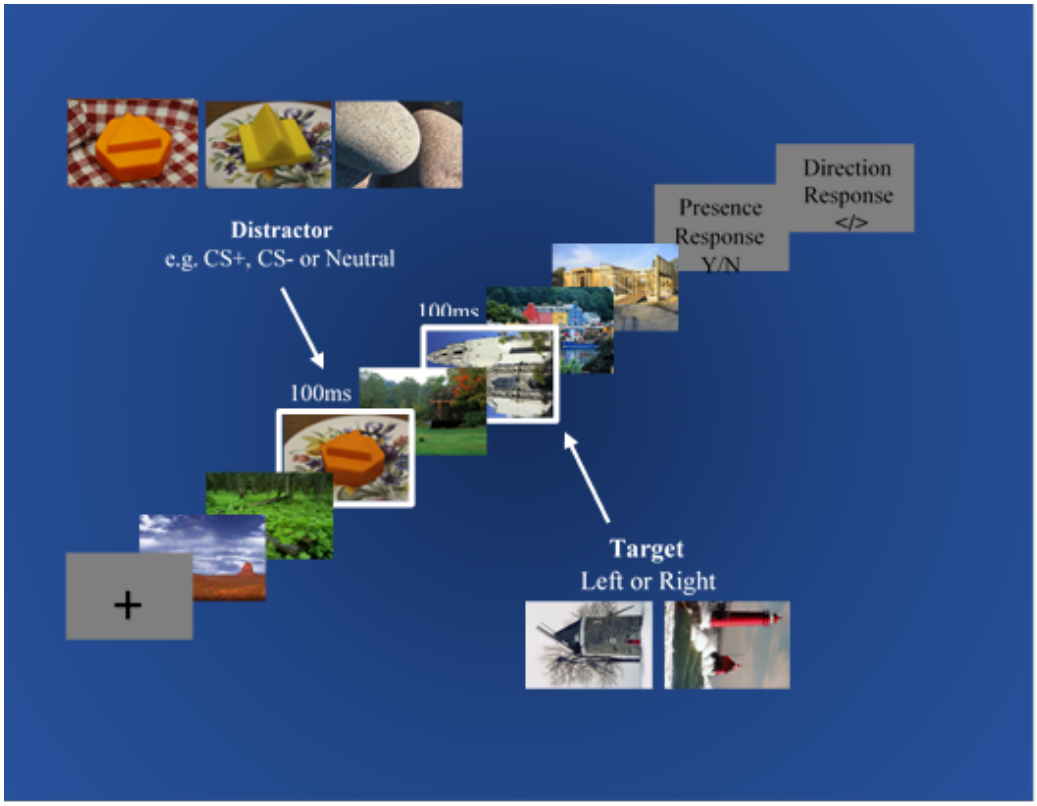


Figure 2.2 Schematic representation of a single trial on the EBA task (Sequence of images has been shortened).

2.5 Overview

Overall, each experiment was based on the same general paradigm (See figure 2.3). Participants were screened prior to beginning the full protocol to ensure suitability. In each experiment, subjective self-report measures of conditioning (e.g. craving,

liking), as well as implicit attentional measures (EBA task) were recorded before and after conditioning in a pre-/post design (neural activity was also recorded using fMRI in Chapter 7). During an acquisition phase participants underwent a novel, naturalistic conditioning procedure, originally developed by Blechert et al. (2016) in which the CS and US form a compound, by instructing participants to eat an edible 3D geometric shape (CS+) and handle a plastic 3D geometric shape in a different colour (CS-). The only exception to this differential procedure was Experiment 3, Chapter 4 which used a between-subjects design. In each experiment participants underwent just one single conditioning trial, except Experiment 4 (Chapter 5) which tracked learning over four conditioning trials. In addition to this, Experiment 5 (Chapter 6) explored whether conditioned responses generalised to stimuli varying in their similarity to the CS+. A battery of questionnaires and additional measures, described above, were completed at the end of each experiment. A full description of each experimental protocol is presented in the respective chapter.

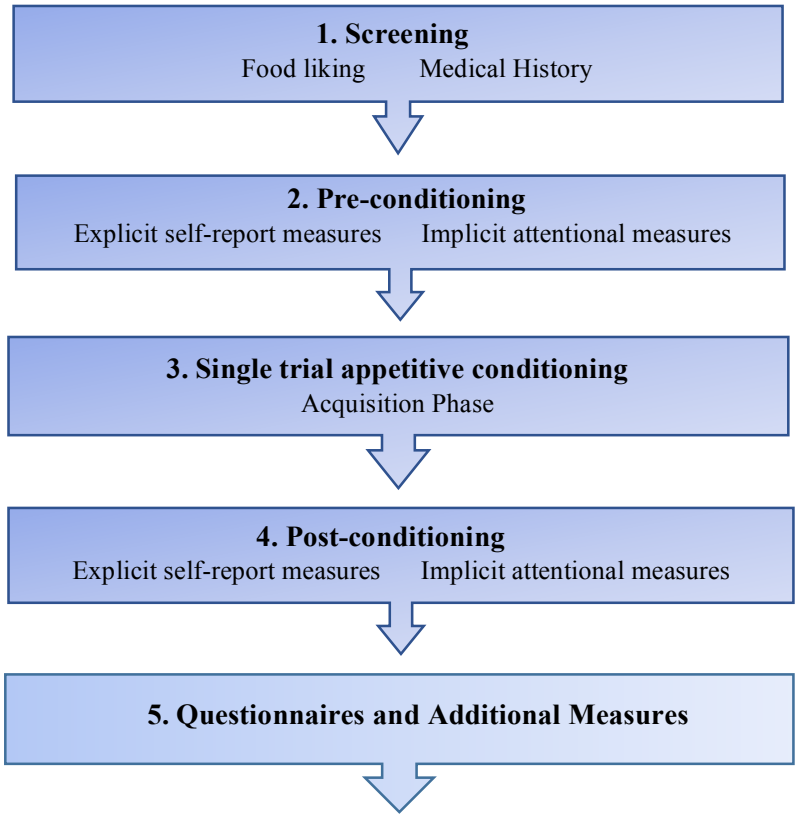


Figure 2.3 Overview of the basic experimental paradigm used throughout this thesis.

Chapter Three

Subjective and attentional markers of single-trial appetitive conditioning.

3.1 Abstract

As outlined in Chapter 1, Pavlovian learning theory suggests that the motivational value of previously neutral cues can be enhanced via associations with reward, resulting in approach motivated behaviours such as cravings. Learned motivational salience can also bias visual attention towards detection of reward-related cues, and may contribute to obesogenic behaviours. However, typical experimental paradigms require many repetitions and lack real-life generalisability to human eating behaviour. Blechert et al. (2016) reported a novel single-trial conditioning technique in which a 'new food' item was created by pairing an unknown geometric object with sweet taste simply by asking the participants to eat the object – made of marzipan. Subsequently, visual presentation of the 'food' objects altered brain activity related to the detection of food, and elicited subjective cravings for sweet foods. A primary aim was to replicate this technique to explore the impact of single-trial conditioning on cravings and attention. Two experiments were conducted which employed this within-subjects single-trial conditioning procedure and measured both subjective responses and a behavioural measure of attention (Emotional Blink of Attention Task (EBA)). Findings from Experiments 1 and 2 suggest conditioning was unsuccessful based on self-report measures. Nonetheless, data from an EBA task suggest that novel cues elicit greater attentional capture than neutral cues post-conditioning, regardless of reward-associations. Potential explanations for this acquired salience are discussed and explored further in chapters 4-7.

3.2 Introduction

There is mounting evidence that reactivity to external food cues can promote overeating, even in the absence of hunger. The mere sight of food can trigger cravings and elicit approach motivated responses, potentially resulting in excessive consumption and weight gain (Tetley, Griffiths & Brunstrom, 2009). This tendency is particularly problematic in today's obesogenic environment where food cues are so abundant.

According to the incentive sensitization hypothesis (Robinson & Berridge, 1993) cues acquire incentive salience through appetitive conditioning. An external cue such as the sight of a fast food restaurant, or the smell of freshly baked bread becomes associated with the ingestion of a delicious food item. Consequently, a once neutral cue becomes a potent signal highlighting the availability of reward, capable of eliciting conditioned responses such as salivation (van den Akker, Schyns & Jansen, 2017) and cravings (Meule et al., 2018).

The majority of studies have successfully demonstrated differential appetitive conditioning: conditioned responses are observed when an appetitive unconditioned stimulus (US; e.g., sweet taste) is paired with a neutral conditioned stimulus, thus predicting reward (CS+), but there is a lack of conditioned responses when a neutral CS is paired with no reward (CS-). Furthermore, conditioning has been successfully demonstrated with a range of palatable food rewards, such as sucrose solution (Franken et al., 2011), chocolate milk (Meyer, Risbrough, Liang & Boutelle, 2015) or salty pretzels (Andreatta & Pauli, 2015).

In most cases, humans readily learn these associations and researchers have measured a variety of conditioned responses to the CS+ but not the CS-. Physiological measures such as heart rate and skin conductance, and explicit self-report ratings, are among the most commonly used measures of conditioned responses (Martin-Soelch, Linthicum & Ernst, 2007).

However, there is a lack of consistency within the literature around the conditions necessary for successful appetitive conditioning in humans. Whereas Franken et al. (2011) used 100 trials to demonstrate conditioning, other studies have used as few as four and there appears to be no clear rationale for trial number. A recent study even highlights the possibility of appetitive conditioning in just a single trial (Blechert et al., 2016). A naturalistic appetitive conditioning procedure was successful in eliciting conditioned cravings for a novel edible object (CS+) but not a

similar, but inedible plastic object CS-. To maximise the strength of conditioning, the CS and US were presented in compound, where both the CS (object appearance) and US (taste of marzipan) were combined, as opposed to the traditional paradigm whereby an image is presented shortly before delivery of a mouthful of food or liquid.

In this paradigm, participants ate an object made from marzipan, of a particular colour and novel, geometric shape. The sweet taste served as a US whereas the novel colour-shape combination served as a CS. They also handled a plastic object which served as a CS-, signalling no reward. Blechert and his team demonstrated that, following conditioning, cravings for sweet foods were elicited by the mere sight of the CS+, but not the CS-, and the CS+ was rated as significantly more pleasant. Additionally, using electroencephalography, the researchers found evidence that this single trial conditioning procedure modulated both early and late neural responses. The authors argue the late neural responses are reflective of the cognitive motivational processing of these cues, whereas modulation of early neural responses represents heightened attentional processing; vigilance for a newly learned food cue was enhanced after just one learning episode.

The tendency for salient appetitive stimuli to preferentially capture and hold attention is referred to in the literature as 'attentional bias' and reflects the current motivational salience of that stimulus (Field et al, 2016). It has been widely demonstrated that humans display an attentional bias towards food cues, particularly those that are palatable or calorific (e.g., Nummenmaa et al., 2011; Gearhardt, Treat, Hollingworth & Corbin, 2012; Toepel et al, 2009), and this heightened vigilance for delicious foods has been implicated in the development and maintenance of obesity (Doolan et al., 2014). However, the factors governing the development and maintenance of these food-related attentional biases are not well understood. Nummenmaa et al. (2011) observed that an attentional bias for food, over non-food, items was only evident when image categories were easily discernible. After close visual matching of food and non-food items, this bias was no longer evident. The authors suggested that, through experience, key visual cues such as shape-colour combinations, may come to be associated with certain foods, thus providing a shortcut for fast, automatic detection of these foods (e.g., a green ball may trigger automatic associations with apples).

A wealth of evidence from the general attention literature seems to support a role for reward learning in determining attentional prioritisation, in a phenomenon termed 'value-driven attentional capture' (Anderson, Laurent & Yantis, 2011a). Anderson and colleagues were the first to show that neutral cues paired with monetary rewards subsequently capture attention even when these cues are irrelevant to the current task and their detection in fact hinders performance. Anderson (2013) argues that this effect represents a distinct attentional network that is critical for human survival: prioritising which stimuli to attend to, and how easily they capture attention, determines whether the acquisition of rewards, and avoidance of punishments is successful. Nonetheless, despite the potential theoretical importance of this work, the experimental paradigms used are typically lengthy, and utilize secondary rewards such as money. Consequently, it is difficult to draw firm conclusions regarding the relevance of these findings to reward-motivated behaviours in the real world, such as eating behaviour and substance-abuse.

Armel et al. (2009) were the first to demonstrate that appetitive conditioning with food rewards could directly influence attentional processing of previously neutral cues. In a preferential looking paradigm, they found that participants looked longer at cues paired with a spoonful of pleasant rather than unpleasant foods. Whilst promising, there are much more accurate and appropriate measures of attention available; results were based on video tapes of participants' eye movements and are reliant of the accuracy of the researchers responsible for coding.

One task which could prove useful for capturing rapid fluctuations in the motivational value of a stimulus is the emotional blink of attention task (EBA). The EBA is an implicit measure of attention which captures the power of an emotionally salient cue to automatically capture attention, even when presented under conditions of limited awareness (McHugo et al., 2013). Piech et al. (2010) demonstrated, using an adapted EBA task, that food cues captured attention to a greater extent when participants were in a fasted state. When the motivational value of food is high, such as under conditions of hunger, food cues are much more potent and capture attention automatically.

Building on this, Davidson, Giesbrecht, Thomas & Kirkham (2018) measured EBA performance at multiple time points preceding and following a meal, and demonstrated that attentional capture fluctuates moment to moment as a function of need state. The implication of this is that implicit measures of attention may serve as

a useful tool for tracking changes in incentive salience of appetitive stimuli, although to date this technique has not been applied to the field of appetitive conditioning.

Single-trial appetitive conditioning warrants further attention: it would seem that even just one learning experience may imbue an otherwise neutral item with incentive salience, eliciting cravings, wanting and approach motivated behaviours. This has significant implications for understanding human eating behaviour, particularly in today's obesogenic environment where we have such an abundance of different food products and marketing strategies, providing endless opportunities for rapid associations to be formed. Understanding the conditions that make this type of learning possible, as well as how this reward learning may influence attentional networks, and subsequent behaviour, will prove important for informing personalised targeted treatments for obesity and developing preventative strategies.

Therefore, the primary aims of Experiment 1 were two-fold:

1. To assess the potential for a naturalistic conditioning procedure to elicit conditioned responses after just a single learning trial, using a replication of the conditioning procedure first described by Blechert et al. (2016). Self-reported cravings and valence (pleasantness) ratings served as explicit measures of conditioning.

2. To explore how single trial conditioning with a novel CS-US food compound may influence attentional capture by a rewarding CS+ (novel food object) and a CS- (novel plastic object) signalling no reward.

In light of the current literature, it was hypothesised that our modified version of Blechert's (2016) naturalistic single trial conditioning procedure would result in conditioned cravings and pleasantness in response to a food-paired CS+ but not a CS- not associated with food.

To explore the second aim, the EBA task was selected as a sensitive measure of a stimulus' current incentive salience, based on the work of Davidson et al. (2018). Following conditioning, it was anticipated that a now rewarding CS+ would automatically capture attention, thus impairing target detection on an EBA task, while visually similar CS- or control images would not be distracting.

3.3 Experiment 1

3.3.1 Methods and Materials

As many of the following methods and materials were used repeatedly across this thesis, they are only described briefly here. Full, detailed descriptions are provided in General Methods (Chapter 2).

3.3.1.1 Participants

Participants were recruited from the University of Liverpool and surrounding area via poster adverts and online adverts on the University website and relevant social media pages. In total 36 participants were tested (12 males). For inclusion, participants were required to be aged 18-40 as selective attention decreases from age 40 onwards, negatively affecting performance on attentional blink tasks (Georgiou-Karistianis et al, 2007). In addition, participants were required to have normal or corrected-to-normal vision, no food allergies or intolerances and not be taking medication known to affect vision, attention or appetite, assessed by a short medical history questionnaire. Participants were also screened before they took part to ensure they liked marzipan. [See Chapter 2 for full description of the screening materials]. Participants were informed that the study was investigating object perception and cognition. This was to avoid highlighting the importance of the different image categories to the experimental aims. Participants gave full informed written consent before participating. The study was approved by the University of Liverpool Ethics Committee.

3.3.1.2 Conditioning

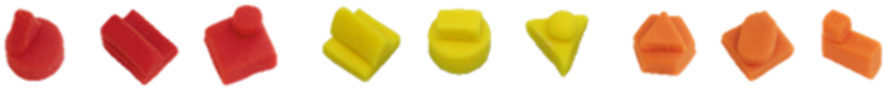
For the conditioning phase, a close replication of the paradigm used by Blechert et al. (2016) was conducted. Participants were presented with three edible objects (CS+), and three plastic objects (CS-). Edible objects were 3D geometric shapes made from marzipan, all produced by a specialist confectioner, of a similar size and shape, each weighing approximately 8 g. The CS- objects were produced by coating marzipan shapes in a plastic resin which gave them a plastic appearance, smell and texture. Three 'unconditioned' objects that were not presented during the conditioning phase were also created, to control for effects of general salience and novelty on attentional capture. Images of these objects were included in the EBA task and were rated for

pleasantness before and after conditioning, but participants never saw or handled physical versions of these objects.

Each object type consisted of three novel combinations of geometric shapes, which were made in three different colours (red, yellow and orange) to allow object colour to be counterbalanced across participants. For example, for a third of participants the CS+ were red, the CS- were yellow and the unconditioned novel objects were yellow (see Table 3.1).

During conditioning, participants were instructed to look at, smell and touch all three CS- objects, and look at, smell, touch and taste the three CS+ objects. They were invited to eat all the edible objects but were informed they could leave them if they wished, in case feeling obligated to eat more than desired minimised the element of reward. All participants were given three minutes to experience both object types, in any order they chose. Participants were also provided with a small glass of water in case they wanted to rinse their mouths after eating the marzipan. Finally, participants were asked to rate the sensory characteristics of the CS+ and CS- stimuli on 100mm VAS scales (See Chapter 2) to ensure participants focused on the objects during conditioning, and to corroborate our cover story that the study was investigating object perception generally. Taste ratings for the CS+ were the only variable of interest.

Table 3.1 Counterbalanced colour-object assignment in Experiment 1.

	Edible Object (CS+)	Inedible Object (CS-)	Visually Similar Control Objects
Group A			
Group B			
Group C			

3.3.1.3 Self-report measures

Appetite

Subjective appetite levels were measured pre- and post-conditioning using four visual analogue scales (VAS). Participants indicated their hunger, desire to eat, fullness and prospective consumption by drawing a vertical mark on a 100 mm line to express the strength of their current feelings. A full description of these scales is provided in Chapter 2.

State Cravings

State cravings, specifically for sweet foods, were assessed using an adapted version of the food craving questionnaire – state version (Cepeda-Benito et al., 2000). This 15-item questionnaire measures subjective state-dependent cravings.

Valence task

Participants completed a short computer task in which they were asked to rate a random sample of five images from each image category of the EBA task for their subjective valence (pleasantness). Participants were asked to rate how pleasant they found each image using 100 mm VAS, with the anchors 'extremely unpleasant' and 'extremely pleasant'. The ratings task was completed once before, and once after conditioning.

3.3.1.4 Attentional measures

Emotional blink of attention task (EBA)

This study adapted the emotional blink of attention (EBA) paradigm previously reported by Davidson, Giesbrecht, Thomas and Kirkham (2018) using a modification of the software generously provided by Dr Graeme Davidson.

As described in Chapter 2, the EBA task involves participants detecting a target image within a rapid serial visual presentation (RSVP) paradigm, presented using E-Prime software. On each task trial, 17 landscape images were presented rapidly on the screen for 100 ms each. A distractor appeared in the fourth, sixth or eighth position in the stream, from one of four distractor categories: CS+, CS-, visually similar controls, or neutral. Distractor categories and positions were counterbalanced across the experiment, and each distractor type appeared 32 times in

total per session. Following each distractor, a target (landscape image rotated 90° degrees either clockwise or counter-clockwise) appeared 200 ms later (lag 2). Immediately after each stream, participants were prompted to indicate, first, the presence of a rotated image ('did you see a rotated landscape image? Y/N') and then its direction ('which way was the image turned? Left/Right'). Responses were classed as accurate when a participant correctly identified both the presence and direction of a target image, reducing the chance level of correct responses from 50% to 25%. Reaction times to each question were recorded automatically by E-prime.

After a practice session, the EBA task was completed pre-conditioning to assess salience of each distractor at baseline. Following conditioning the EBA task was completed for a final time. Each EBA session included 4 blocks of 32 trials (128 trials in total), with a rest interval of one minute between each block.

Task stimuli

All task stimuli were colour images, resized to 320 x 240 pixels, and matched for luminance. Filler images were photographs of natural landscapes, such as trees and mountains and urban scenes, selected from a total bank of 252 images. A subset of these images served as the targets and were rotated either 90° clockwise (135) or counter-clockwise (136). For the neutral distractor category, 48 images were randomly selected from the International Affective Picture System (IAPS) database (Lang, Bradley, & Cuthbert, 2001). These were everyday images that are consistently rated as having neutral valence and arousal.

To create the CS+, CS- and control stimuli, photographs were taken of the 9 different objects against different backgrounds, to reduce the salience and contrast between these stimuli and the landscape and neutral images. Backgrounds were neutral and consisted of different everyday household settings. In total 32 images were selected for each of the three novel object conditions.

3.3.1.5 Questionnaires and additional measures

The questionnaires used in this experiment are described in detail in General Methods (Chapter 2). Participants completed the Dutch Eating Behaviour Questionnaire (DEBQ; Van Strien, Frijters, Bergers & DeFares, 1986) and the Food Neophobia Scale (FNS; Pliner & Hobden, 1992) to provide descriptive statistics about eating behaviour and attitudes to food in the current sample.

3.3.2 Procedure

An overview of the study procedure is illustrated in figure 3.1. Participants arrived at the lab between 11am and 5pm. Prior to arrival, participants were instructed to refrain from eating or drinking for at least two hours to ensure participants had not recently eaten. This timeframe was chosen to create a mild to moderate state of hunger that individuals experience as they approach their next meal. Longer periods of deprivation such as an overnight fast used in other conditioning research (e.g. Andreatta & Pauli, 2015) were considered unnecessary for the present aims and may have drawn attention to the experimental aims. Upon arrival at the lab, participants were seated in individual booths and completed the screening. Eligible participants went on to provide baseline measures of hunger and state cravings. After successful completion of the practice EBA, participants completed the pre-conditioning EBA session followed by the valence VAS task. Once all baseline measures were completed, participants underwent the conditioning phase of the experiment: the CS+ and CS- were sampled for 3 minutes and then participants rated their sensory characteristics. Post-conditioning, participants repeated self-reported hunger and craving measures, repeated the EBA task, and again completed the valence task, followed by the DEBQ and FNS. At the end of the study, contingency awareness was assessed by asking participants to recall the colours and shapes of the CS+ and CS-. This was done to ensure participants were aware of the relationship between the objects' visual appearance and its rewarding properties. Participants were also asked to write down their beliefs about the experimental aims to ensure they believed the cover story. Finally, height and weight were measured using calibrated scales and a stadiometer. Participants received a full written debrief and either a £15 high street voucher or course credit as compensation for their time and effort. In total, the full experimental procedure took approximately 75 minutes to complete.

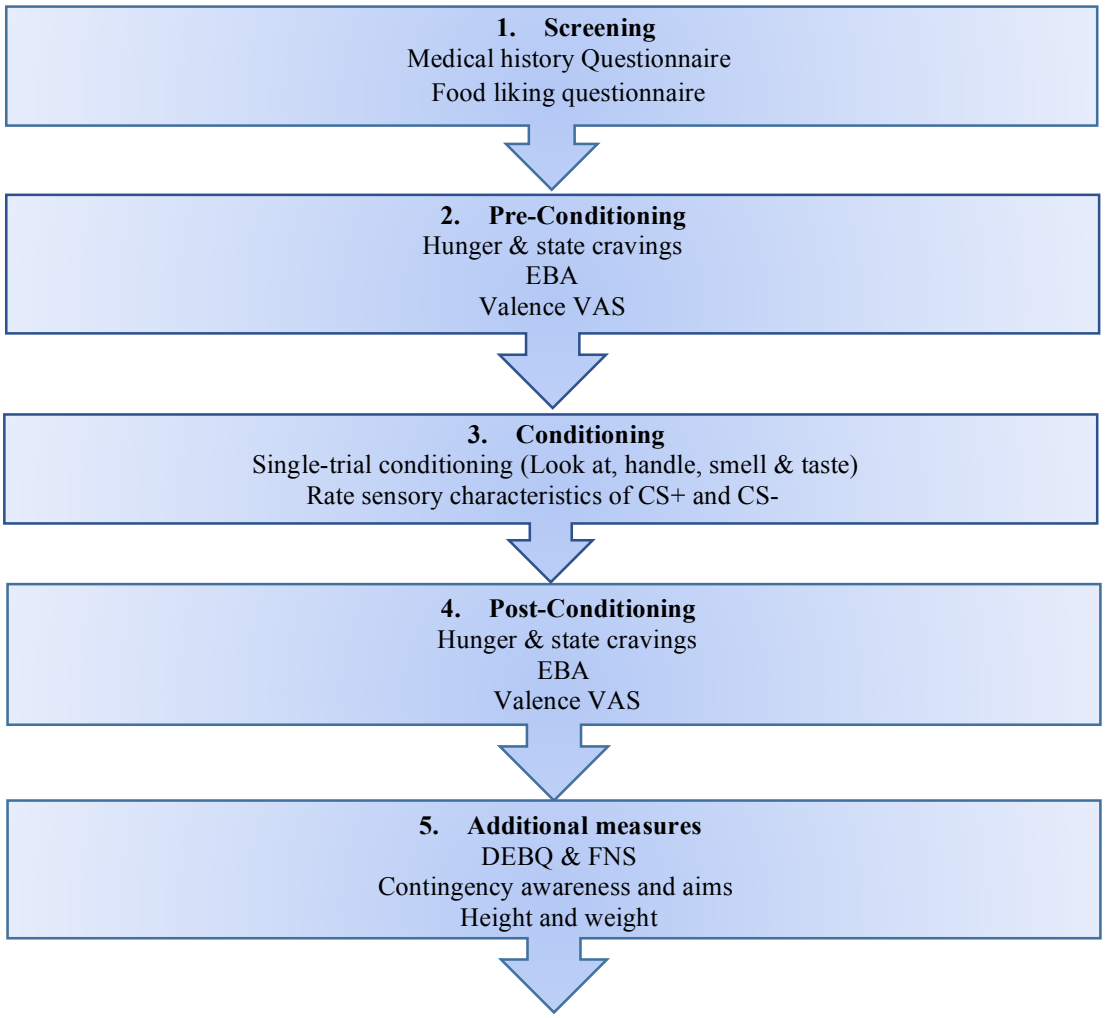


Figure 3.1 Flow chart depicting the study procedure for experiment one.

3.3.3 Data preparation and analysis

3.3.3.1 EBA task

On the EBA task, responses were categorized as correct where participants correctly identified both the presence and the direction of the target. This reduced the probability of correct responses occurring by chance to just 25%. Additionally, average reaction times were calculated and trials where responses were more than 2.5 standard deviations outside the mean were also classed as incorrect, in line with similar research investigating attentional processes (Anderson, 2017). The process of excluding particularly fast or slow trials is common practice in reaction time tasks as these do not reflect typical responding; fast responses may be initiated before the participant has processed the stimuli, whereas slow responses may represent a lack of

concentration or guesswork (Anscombe, 1960). The percentage of correct responses, and the average reaction times for trials with each image category were calculated based on the remaining data.

3.3.3.2 Statistical analysis

IBM SPSS Statistics version 24 was used to analyse the data. All data are presented as mean ± SD unless otherwise stated. Within-subject responses on computerized tasks (EBA and valence task) were examined using repeated measures ANOVA on two levels (time × distractor type). Performance on the EBA task was operationalized as the percentage of trials for each distractor category in which the target was correctly identified. Lower accuracy on the task indicates a greater degree of attentional capture. Where the assumption of sphericity was violated, results are reported with Greenhouse-Geisser corrections applied. Where significant interaction effects were identified, simple effects were explored and followed up using Bonferroni adjusted pairwise comparisons. Relationships between task performance and individual differences were explored using Pearson's product moment correlation coefficient. Statistical significance was accepted at $p \leq 0.05$.

3.3.4 Results

3.3.4.1 Participant characteristics

Table 3.2 provides an overview of participant characteristics. Data from one participant was excluded due to consistently poor accuracy on the EBA task and very few correct trials overall; total accuracy fell more than 2.5 SDs below mean performance rates. In addition, two participants were assigned incorrect colour-shape combinations due to experimenter error and removed from all analysis. Therefore, the following results refer to the remaining 33 participants.

Participants were aged 19 - 40 and on average a healthy BMI. In the present sample, levels of food neophobia were similar to that reported in a comparable sample of European university students (Fenko, Leufkens & van Hoof, 2015; mean ± SD: 29.39 ± 10.07). Scores ranged from 11 - 43 and, using the cut offs proposed by Previato and Behrens (2015), 64% of the sample can be classified as neutral (16.5-38.5), 27% as Neophillic (<16.4) and just 9% as neophobic (>38.6). Eating attitudes were also consistent with mean scores of a sample of non-clinical Dutch

undergraduates on DEBQ subscales restraint, emotional eating and external eating (2.47 ± 0.88; 2.48 ± 0.71; and 3.13 ± 0.51 respectively) (van Strien, Peter Herman & Anschutz, 2011).

All participants were able to accurately recall the CS-US contingencies. Each participant correctly recalled the colour assignment of the CS+ and CS- and were able to describe the shape of the CS+ and CS- objects. Only one participant identified the aims of the experiment with any degree of accuracy and their exclusion from analysis did not alter the pattern of results.

Table 3.2 Participant characteristics (Mean ± SD)

Variable	***M (±SD)***
Gender	
Female N (%)	21 (63.6%)
Age	27.24 (*5.56*)
BMI	24.46 (*4.87*)
DEBQ	
Restraint (α = .87)	2.40 (*.74*)
Emotional (α = .89)	2.42 (*.97*)
External (α = .96)	3.37 (*.70*)
FNS (α = .87)	24.55 (*9.40*)

BMI = body mass index, DEBQ = Dutch Eating Behaviour Questionnaire; FNS = Food Neophobia Scale. As the DEBQ has a distinct three-factor structure, a total score was not Computed.

3.3.4.2 Checking Assumptions

Visual inspection of Q-Q plots based on standardized residuals suggested the data were approximately normally distributed. Consequently, parametric tests were conducted and data is presented with no transformations applied

3.3.4.3 Counterbalancing

After counterbalancing for colour-object assignment, each group (A, B or C) were evenly matched on all variables (*p*s > .05). Mixed ANOVAs revealed no effect of group on any variable of interest, suggesting that CS colour and shape had no influence on conditioning. Therefore, this factor was dropped from all analyses.

3.3.4.4 Emotional Blink of Attention (EBA)

Response Accuracy

A 2 × 4 within-subjects ANOVA was conducted to explore the effect of time (pre- and post-conditioning) and distractor type (CS+, CS-, Novel Control Object and Neutral) on attentional capture. This revealed a significant main effect of distractor type, $F(3, 96) = 2.96$, $p = .036$, $\eta_p^2 = .09$, and a time × distractor type interaction, $F(3, 96) = 3.60$, $p = .016$, $\eta_p^2 = .10$ (see Figure 3.2). Follow-up analysis was focused on the interaction term.

Contrary to our predictions, the simple effect of time was not significant for any distractor type (*p*s ≥ .10), suggesting that accuracy for each distractor was not significantly affected by the conditioning procedure. In order to fully explore the interaction, the simple effect of distractor type at each time point was explored. As anticipated there were no differences in accuracy between distractor types pre-conditioning; $F(3, 96) = 1.45$, $p = .232$, $\eta_p^2 = .043$; all distractors were equally salient. However, post conditioning, the simple effect of distractor type was significant; $F(2.33, 74.45) = 5.17$, $p = .005$, $\eta_p^2 = .14$.

As predicted, after single-trial conditioning, the CS+ objects captured attention to a greater extent than the neutral distractors, $p = .027$. However, contrary to our predictions, CS- distractors were also more distracting than neutral, $p = .012$. The difference between correct responses for neutral distractor trials and novel control object trials trials failed to reach significance, $p = .214$.

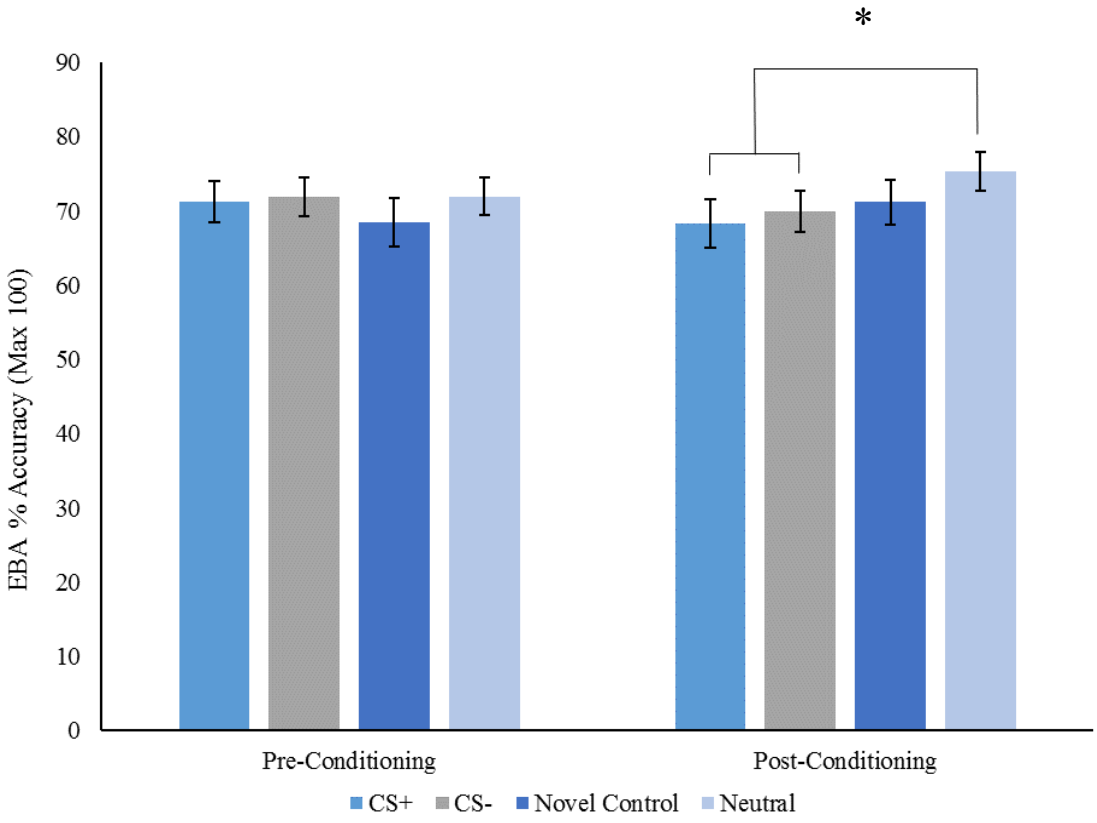


Figure 3.2 Percentage of accurate target detection on the EBA task for trials with each distractor category, pre- and post-conditioning. ** p<.05, ** p<.01, *** p<.001*

Reaction times

A 2 × 4 repeated measures ANOVA revealed a significant main effect of time, but no effect of distractor type or a time × distractor type interaction. Reaction times got quicker from pre- (301.88, 112.00*)* to post- (258.49 ± 88.79) conditioning ($F(1, 32) = 18.22$, $p = .001$, $\eta_p^2 = .363$) but did not differ between distractor type, most likely representing a simple practice effect.

3.3.4.5 Valence Ratings

There was a significant main effect of image type (CS+, CS-, Novel Control, Neutral, Filler), $F(1.43, 45.87) = 49.34$, $p = .001$, $\eta_p^2 = .61$, and a significant interaction between time (pre- and post-conditioning) and image type on valence ratings, ($F(2.83, 90.64) = 4.89$, $p = .004$, $\eta_p^2 = .133$)

Overall, images of the CS+ (41.55 ± 15.27), CS- (40.72 ± 14.88) and novel control objects (40.25 ± 13.59) were rated as significantly less pleasant than neutral (60.44 ± 9.05) and filler images (70.38 ± 11.04) at both time points, *p*s ≤ .001.

Post-hoc comparisons were conducted to explore the interaction term (see Figure 3.3). These revealed that the interaction was driven by a slight increase in valence ratings for the CS+ from pre- to post conditioning (p = .01) and a decrease in valence ratings for the landscape filler from pre- to post-conditioning (p =.002). Ratings for the other image categories did not change (*p*s >.05).

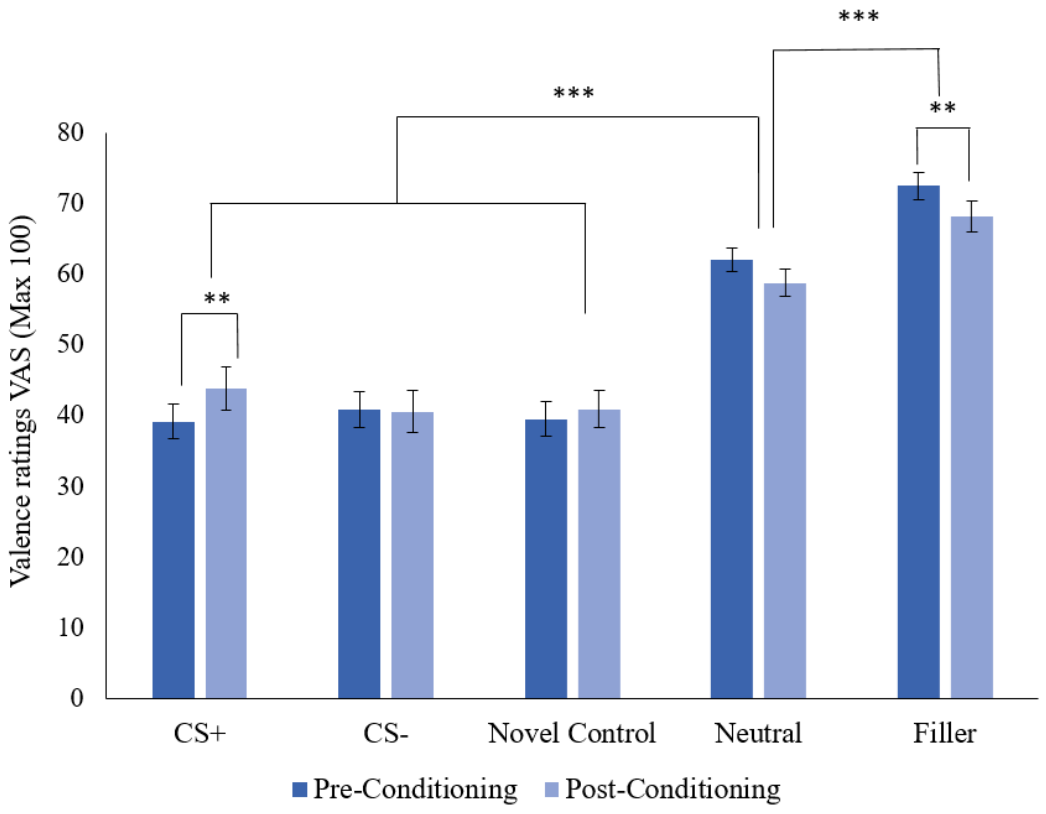


Figure 3.3 Average valence ratings for each image category pre- and post-conditioning. Error bars represent standard error. ** p<.05, ** p<.01, *** p<.001*

3.3.4.6 Conditioning: taste and consumption

Taste ratings for the CS+ measured during acquisition showed the marzipan objects were generally well liked (62.76 ± 22.51) but with a large spread of scores (Min = 11, Max = 95). 21 participants (64%) provided taste ratings above the mean. On average, participants consumed 27.95g (± 3.19) of marzipan during the experiment.

All participants ate a minimum of 60% of the marzipan presented with 82% of participants choosing to consume all of it. The amount of marzipan consumed was positively correlated with taste ratings, r_s (31) =.53, p = .002. The more participants liked the marzipan taste, the more likely they were to finish it all.

3.3.4.7 Hunger and state cravings

Change in self-reported hunger from pre- to post-conditioning was assessed using a paired-sample t-test. Hunger significantly decreased from pre- (66.06 ± 21.31) to post-conditioning (56.23 ± 20.99), t(32) = 4.22 , p < .00, d = .73.

Due to a technical error, responses for two of the questions on the FCQ-S were not recorded. Therefore, scores represent responses to the first 13 items on the scale only.

A paired-sample t-test showed that cravings for sweet foods were significantly higher before conditioning (40.58 ± 8.85) than after (34.67 ± 9.18), t(32) = 4.81, p = .001, d = .84.

Exploratory analysis was conducted to determine whether the amount of marzipan consumed (g) or taste ratings for the CS+ were associated with the degree of change in self-reported hunger or cravings across the experiment. No associations were found between marzipan consumption (g) and pre-post difference in hunger ratings, r_s (31) =.-.07, p = .681 or cravings, r_s (31) =-.01. p = .944. Furthermore, there was no association between taste ratings and pre-post change in hunger, r_s (31) =.-.15, p = .393 or cravings, r_s (31) =-.07, p = .686.

3.3.4.8 Associations with individual differences

Pearson's correlational analysis was conducted between pre-post difference scores (post-conditioning minus pre-conditioning) on outcome measures (EBA, valence, cravings) and individual differences (DEBQ, FNS, BMI, age). The correlation matrix is presented in Table C1, Apprendix C. No significant correlations were identified.

3.3.5 Interim Discussion

Experiment 1 was designed to examine the potency of a novel, naturalistic appetitive conditioning procedure, first described by Blechert et al. (2016), for imbuing an otherwise neutral cue with incentive salience after just one appetitive conditioning trial. Implicit attention (EBA task) and explicit self-report ratings (cravings and

valence), were measured both before and after participants ate edible, novel objects made of marzipan (CS+) or handled inedible plastic objects (CS-). In this naturalistic procedure the CS+ represented the sight of food, whilst the sweet taste of marzipan served as an appetitive US, mimicking a natural encounter with a new food from initial sight, through to consumption.

Contrary to expectations, evidence for appetitive conditioning on self-report measures, was lacking. In fact, cravings for sweet foods were lower after conditioning than at baseline. Conditioned cravings for a food-paired cue develop readily and are a key marker of a cue's incentive salience (Havermans, 2013). Thus, it is questionable whether differential appetitive conditioning was in fact successful after this single-trial conditioning episode. Furthermore, although CS+ ratings on a valence task increased slightly after conditioning, this change was minimal and, overall, these objects were still rated as less attractive than neutral images and landscape scenes. These results are in direct contrast to those of Blechert et al. (2016) who successfully demonstrated robust differential appetitive conditioning on self-report measures as well as ERPs. It would seem that in this study a single trial was insufficient to transform the CS+ in to a highly valued and craved food cue.

When considering implicit attention, some small yet significant effects were observed. Prior to conditioning, images of the novel marzipan (CS+) and plastic (CS-) objects captured attention to the same degree as visually similar control objects and neutral distractor images, thus reducing the possibility that low-level visual features such as brightness, or simply the novel appearance of the shapes impacted on performance. In line with our original predictions, the CS+ was apparently more distracting than neutral images after conditioning. However, the same pattern also emerged for the plastic-paired objects, despite participants demonstrating explicit awareness that these cues were inedible. Although the difference between the novel, unhandled objects and neutral distractors failed to reach statistical significance, it is worth noting that there was no significant difference in accuracy between post-conditioning CS+, CS- and novel control trials, overall.

There are a number of design features associated with the present experiment that warrant consideration, and which may have contributed to these erroneous results. For example, the conditioning procedure involved the simultaneous presentation of the CS+ and CS- objects in one three minute episode, which may have enabled learning to spread, in a phenomenon known as second order

conditioning. This form of learning occurs when a second conditioned stimulus (CS2) begins to elicit a conditioned response after pairing with the first order conditioned stimulus (CS1), even though the CS2 and US were never directly paired (e.g., Holland, 1981). So in this instance, participants may have learned an association between the CS+ (CS1) and the CS- (CS2) so that both captured attention despite the CS- and US never being explicitly paired. A carefully designed conditioning procedure, which successfully separates the CS+ and CS- in order to prevent such learning from taking place would be important to exclude this possibility.

However, this still does not account for the apparent lack of conditioning on self-report measures. As discussed, cravings for sweet foods decreased following conditioning, and a decrease in subjective hunger was also observed. Baseline self-report ratings indicate that participants arrived at the lab in a state of moderate hunger and ate a minimal amount of food (no more than three small marzipan sweets totalling less than 30g). Therefore, this change is unlikely to represent true satiation. A more plausible explanation is that reduced appetite represents an effect known as sensory specific satiety (SSS). Current evidence suggests that a food's incentive salience is temporarily reduced when eaten to satiety; the pleasantness of a particular food decreases with its consumption, relative to other uneaten foods differing in their sensory qualities (e.g., Wilkinson & Brunstrom, 2016). This SSS is independent of calories ingested and can occur even if a food item is merely chewed and not swallowed (Rolls & Rolls, 1997). Therefore, although participants were likely not physically satiated by such a small amount of food, the taste and smell alone may have significantly decreased the pleasure experienced by eating the CS+. No relationships emerged between the change in hunger and craving scores pre- to post-conditioning taste ratings and amount of marzipan consumed, although this is likely due to a lack of variation between scores.

Sensory specific satiety has been shown to modulate attentional processing of food cues. Davidson, Giesbrecht, Thomas & Kirkham (2018) observed a reduction in attentional blink magnitude when images of a specific food eaten to satiety are presented as distractors in the EBA. In fact, patterns of brain activity related to approach motivation have been found during eating when hungry, but this switches to a pattern characteristic of avoidance motivation as eating continues to the point of becoming unpleasant (Small, Zatorre, Dagher, Evans & Jones-Gotman, 2001). Thus,

despite slightly enhanced salience for the novel objects post-conditioning, the occurrence of SSS across the experiment may have minimised the expression of strong motivational salience by the CS+. Additionally, SSS may be stronger for foods with strong, rather than weak, taste intensity (Snoek, Huntjens, van Gemert, de Graaf & Weenen, 2004) and, as marzipan is highly sweet with a characteristic, strong almond flavour this may have led to more rapid SSS. This may explain why the anticipated reduction in EBA accuracy for the CS+ relative to the other image categories was not observed: in effect, although there may have been successful appetitive conditioning, SSS induced by the CS+ may have reduced its incentive value and so opposed the ability of the EBA to evidence that learning.

A further limitation of the present experiment is that comparative attentional capture by food, or other rewarding cues, with a rich learning history was not measured. Thus, it is not possible to determine how a small change of attentional capture by CS+ relative to neutral distractors would compare to attention to rewarding cues, which have been consumed on many occasions and are easily recognisable. Future work may benefit from exploring how reward history influences learning and attention processes. Experiment 2 was designed to address these limitations to provide a more thorough test of single trial appetitive conditioning.

3.4 Experiment 2

3.4.1 Method

3.4.1.1 Participants

Recruitment and screening were conducted as described above. Participants in Experiment 1 were excluded from participation in this experiment due to the similarity in aims and protocol. A total of 35 participants were recruited from the University of Liverpool and surrounding area. Participants gave full informed written consent before participating. The study was approved by the University of Liverpool Ethics Committee.

3.4.1.2 Conditioning

Again, a naturalistic appetitive conditioning paradigm was employed, revised from Experiment 1 to prevent the development of SSS, and to reduce design complexity. This modified procedure incorporated a single novel edible object (CS+), made of marzipan, and a single novel object coated in plastic (CS-), so only 10g was consumed during the experiment. Each could be a specific shape and colour (yellow or orange), and again colour-shape assignment was counter-balanced across participants (See Table 3.3). Data from Experiment 1 indicated that the shape of each object had no influence on attention or valence so the two shapes used here were selected randomly. Colours yellow and orange were chosen rather than red as some evidence suggests red may have an enhanced ability to guide attention (Kunjecki, Pilarczyk & Wichary, 2015), although our initial data did not appear to support this.

Furthermore, in order to remove the possibility of second order conditioning, the conditioning procedure was adapted so that the CS+ and CS- were never presented at the same time; instead, each stimulus was presented singly, in a counterbalanced order. For half the participants the CS+ was presented first on a white plate and they were asked to look at, touch, handle, smell, and taste the marzipan object over a period of two minutes. They then rated the sensory characteristics on 100 mm VAS scales. After a break of approximately 90 seconds the CS- was then presented in a white plastic box, and participants were asked to look at, handle and smell the plastic object

Table 3.3 Counterbalancing of colour-object assignment in experiment two.

	Stimulus Type	
Group	CS+	CS-
1		
2		

3.4.1.3 EBA task

The EBA task design and procedure was largely consistent with Experiment 1 with minimal changes to the distractor categories and images. Again, there were four distractor categories in total, but this time images of the unhandled abstract objects were replaced with images of highly palatable desserts. The final four distractor categories were: CS+, CS-, Dessert and Neutral.

The inclusion of dessert images was based on the observation that desserts appear to be particularly salient and desirable: images of desserts, such as a rich chocolate cake, reliably capture attention regardless of participants' current motivational state (Davidson, et al., 2018). Consequently, the inclusion of these images would allow comparisons to be made between the incentive salience of a newly learned appetitive CS and well-known food cues with a strong reward history.

A new set of photographs was taken of the two selected conditioning objects, in a similar fashion to Experiment 1; 40 CS+ and 40 CS- images were selected for use in the experiment. The objects were photographed against various neutral backgrounds and matched for luminance and complexity with the other image categories. Forty-eight images of desserts were selected from a set of images used by Davidson et al. (2018). All images in the task were colour photographs, resized to 320 × 240 pixels.

3.4.2 Procedure

Participants were asked to refrain from eating or drinking calorific food or beverages (water was excluded) for two hours prior to participation. Participants arrived at the lab between 11:00 and 14:00. They were informed that the study was investigating object perception and hunger. Eligible participants provided full informed consent and completed the revised experimental procedure – as outlined for Experiment 1, with the adapted conditioning stage (see 3.9.2, above) and modified EBA content (3.9.3).

3.4.3 Results

3.4.3.1 Participant Characteristics

One participant exhibited particularly poor performance on the EBA task; overall accuracy was less than 25% which is below chance level, and thus this participant

was removed from all analysis. Results are presented for the final 34 participants. Participant characteristics are shown in table 3.4. Levels of food neophobia were consistent with those in Experiment 1 and ranged from 10 – 45. Using the cut offs proposed by Previato and Behrens (2015), 67% of the sample can be classified as neutral, 26% as Neophillic and 6% as neophobic. Mean scores across the three DEBQ subscales were slightly elevated in comparison to non-clinical norms (van Strien, Herman & Anschutz, 2011). No significant outliers were identified.

MANOVA confirmed that participant characteristics did not differ by group (colour-object assignment), $F(7, 26) = 1.76$, $p = .140$, Pillai's trace = .321, $\eta_p^2 = .32$. The ratio of males to females was exactly the same in both groups (10 females, 7 males). Mixed ANOVAs revealed no effect of group on any of our dependent variables, so this factor was dropped from all subsequent analyses. This would suggest that CS colour and shape had no influence on implicit or explicit conditioning processes. All participants accurately recalled the CS-US contingencies and no participant accurately guessed the aims of the experiment.

Table 3.4 Participant characteristics (Mean ± SD).

Variable	***M (±SD)***
Gender	
Female N(*%*)	20 (*59%*)
Age	24.02 (*5.98*)
BMI	24.14 (*3.72*)
DEBQ	
Restraint (α = .89)	2.60 (*.66*)
Emotional (α = .95)	2.81 (*.88*)
External (α = .74)	3.52 (*.44*)
FNS (α = .88)	23.50 (*8.87*)

BMI = body mass index, DEBQ = Dutch Eating Behaviour Questionnaire; FNS = Food Neophobia Scale. As the DEBQ has a distinct three-factor structure a total score was not Computed.

3.4.3.2 Self-report data

Appetite and cravings

Self-reported appetite remained unchanged across the experiment (Table 3.5), suggesting our procedure modification was successful in preventing marked changes in appetite or the induction of sensory specific satiety. Self-reported cravings for sweet foods also remained the same at both time-points, suggesting that, as in experiment one, the single trial conditioning procedure was unsuccessful at producing conditioned cravings.

Table 3.5 Results of paired t-tests comparing self-reported hunger and cravings pre- and post-conditioning.

	Pre-conditioning		Post-conditioning		t-test		
	M	*SD*	*M*	*SD*	*t*	*p*	*d*
Appetite	69.53	15.38	65.47	19.82	1.61	.12	.28
Cravings	45.12	7.66	45.14	10.62	.019	.99	-.01

3.4.3.3 Conditioning: Taste ratings and consumption

All participants consumed the whole object (10.72g ± .85). The taste of the CS+ object was generally well liked (61.35 ± 26.74); 65% of participants provided taste ratings above the mean. However, there was a wide spread of scores (Min = 0, Max = 92). Five outliers were identified who provided particularly low ratings but their exclusion did not alter the overall pattern of results. Consequently, to maintain power their data was kept in the final analysis.

3.4.3.4 Subjective valence ratings

A 2 × 5 repeated measures ANOVA revealed a significant main effect of image type, $F(2.64, 81.30) = 75.13$, $p < .001$, $\eta_p^2 = .70$, and a significant interaction between time and image type, $F(4, 132) = 3.64$, $p = .008$, $\eta_p^2 = .10$. In line with our hypotheses, follow-up analyses were conducted to further explore this interaction.

Overall, dessert images (67.24 ± 12.87) and filler images (63.89 ± 9.53) were rated more attractive than all other image types, regardless of time point, whilst the CS+ (37.99 ± 11.75) and CS- (35.58 ± 10.66) images were rated as least attractive overall (*p*s < .001).

Paired t-tests were conducted to explore the change in valence ratings over time (see Figure 3.4). This revealed that valence ratings for dessert, CS+ and CS- images did not change from pre- to post-conditioning (*p*s ≥.103). There was, however, a significant decrease in valence ratings for the neutral images from pre- (56.96 ± 10.30) to post-conditioning (50.62 ± 12.61), driving the interaction term, $t(33) = 3.22$, $p = .003$, $d = .55$. The decrease in valence ratings from pre- (66.34 ± 9.88) to post-conditioning (61.43 ± 12.35) for the landscape filler images did not survive a bonferroni correction for multiple comparisons, $t(33) = 2.44$, $p = .020$, $d = .42$.

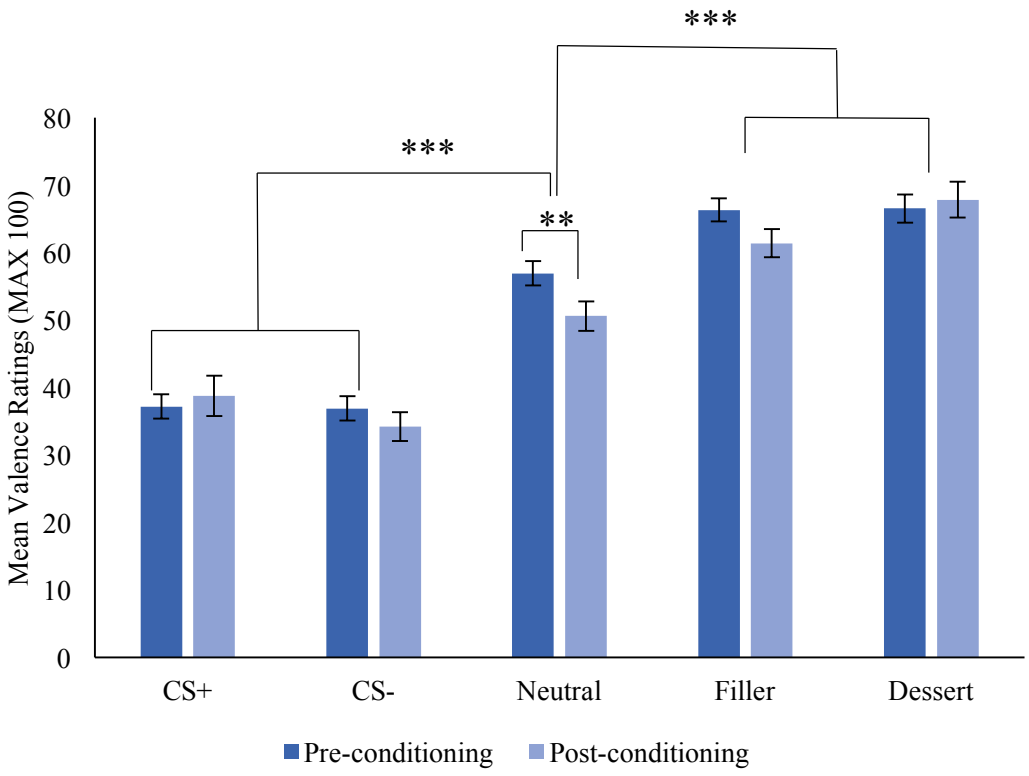


Figure 3.4 Mean valence ratings of a random sample of images from the EBA task, measured on 100 mm VAS, both before and after conditioning. ** p<.05, ** p<.01, *** p<.001*

3.4.3.5 EBA performance

Percentage accuracy

A 2 × 4 repeated measures ANOVA revealed a significant main effect of distractor type $F(3, 99) = 3.30$, $p = .023$, $\eta_p^2 = .091$, and a time × distractor type interaction on percentage of correct responses on the EBA task, $F(3, 99) = 2.85$, $p = .041$, $\eta_p^2 = .079$ (see Figure 3.5).

Paired t-tests were conducted to explore the simple effect of time on each distractor type. Again, accuracy on trials with a CS+, CS- or dessert distractor present failed to change from pre- to post-conditioning (*p*s ≥ .276). Accuracy on trials with neutral distractors improved from pre- to post-conditioning, $t(33) = 2.40$, $p = .022$, $d = 0.41$. Although, this failed to survive a bonferroni correction.

As anticipated, the simple effect of distractor type failed to reach significance prior to conditioning ($p > .05$); performance was similar on all trials regardless of distractor type. However, the simple effect of distractor type was significant post-conditioning, $F(2.41, 79.63) = 5.45$, $p = .004$, $\eta_p^2 = .142$. Post-hoc comparisons revealed that CS+, CS- and dessert distractors all captured attention more than neutral distractors after conditioning (*p*s ≤ .031).

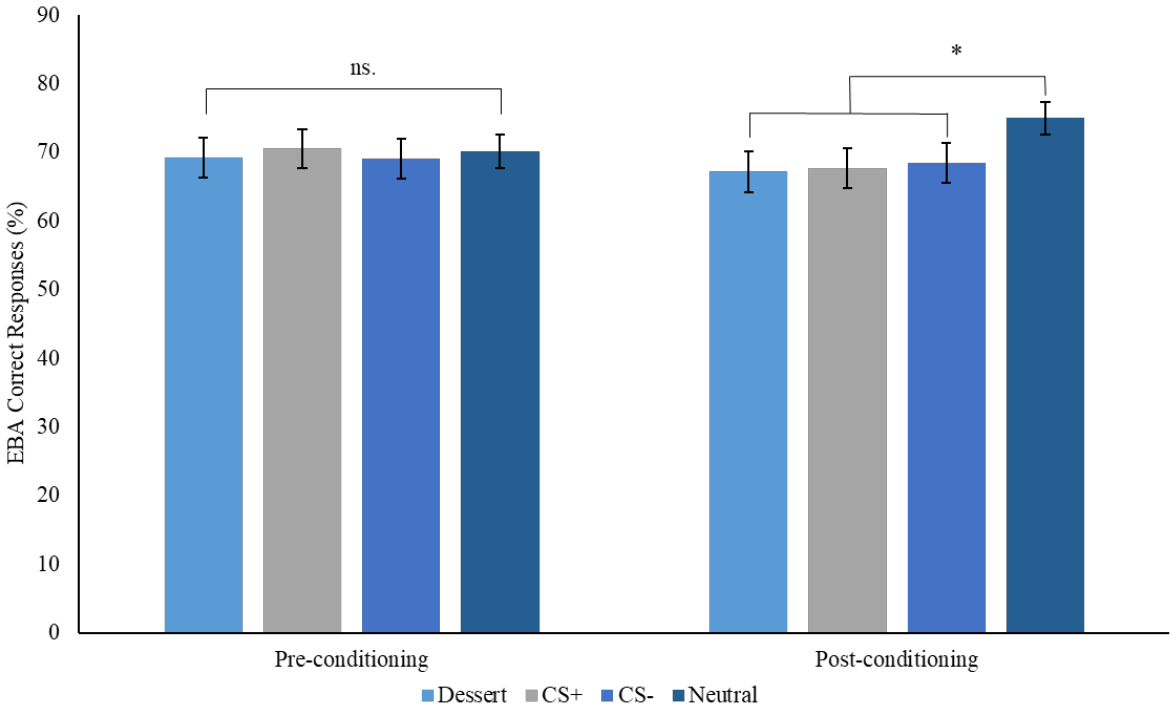


Figure 3.5 Percentage of correct responses on EBA trials with the different distractor types, before and after single-trial conditioning (M ± SE). ** p<.05, ** p<.01, *** p<.001*

Reaction times

A 2 × 4 repeated measures ANOVA revealed a significant main effect of time, $F(1, 33) = 49.26$, $p = .001$, $\eta_p^2 = .599$. The main effect of type and the interaction term failed to reach statistical significance. Overall reaction times improved significantly from pre-conditioning (270.84 ms ± 109.32) to post-conditioning (221.85 ms ± 85.96) ($p < .001$), most likely due to task familiarity.

3.4.3.6 Exploratory analysis

Associations between taste ratings and conditioning

In an attempt to understand why clear evidence of conditioned responses did not emerge, some exploratory analysis was conducted. Change (difference) scores for CS+ valence ratings from pre- to post-conditioning were calculated. Pearson correlation indicated that there was a weak, yet significant correlation between the degree of change in perceived pleasantness of the CS+ images, and the CS+ taste ratings, $r(32) = .39$, $p = .022$ (see Figure 3.6). The more participants enjoyed the taste of marzipan, the greater the change in valence scores for the CS+ images from pre- to post-conditioning. Taste ratings of the CS+ were unrelated to the degree of change in other measures of conditioning (EBA accuracy on CS+ distractor trials, or change in conditioned cravings).

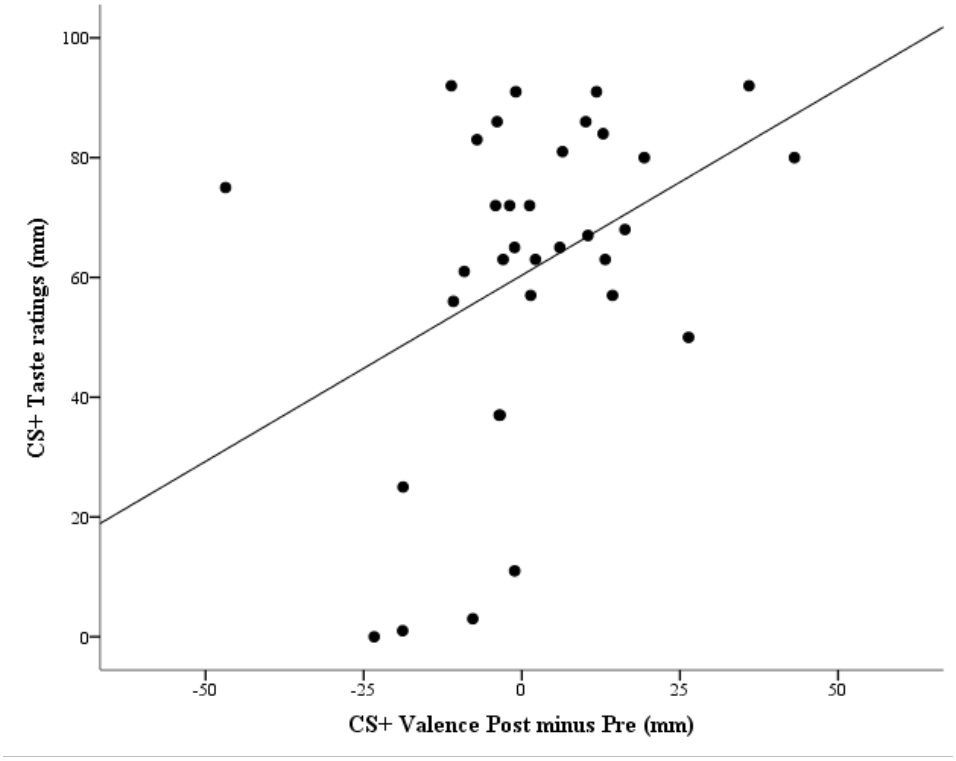


Figure 3.6 Exploratory correlation between CS+ taste ratings and the difference score in CS+ valence ratings (post- minus pre-conditioning). Note: CS + = image of novel object that was associated with edibility and marzipan taste during single-trial conditioning.

Associations with individual differences

Correlations were conducted between pre-post difference scores on outcome measures (EBA % Correct Responses, VAS valence scores and cravings) and individual differences. (See Table C2, Appendix C). After applying corrections for multiple comparisons, no significant correlations emerged.

3.4.4 Discussion

Experiment 2 was conducted to resolve several potential limitations highlighted for Experiment 1, and further explore the ability of a naturalistic one-trial appetitive conditioning paradigm to transform a novel object in to a highly likable, craved stimulus, capable of modulating involuntary attentional capture. The results showed that, despite the modifications, evidence for single trial conditioning on self-report

measures was still lacking. Subtle differences in implicit attention were apparent from pre- to post conditioning: before conditioning, all distractors were equally salient, yet images of CS+, CS- and highly palatable dessert images captured attention to a greater extent than neutral images after a naturalistic single trial conditioning procedure. Exploratory analysis suggests that low reward value of the CS+ may account for the apparent lack of clear conditioned responses.

In the present experiment, participants underwent a modified version of the original paradigm; this time they were asked to eat just one edible food object made of marzipan (CS+) and handle one inedible object made of plastic (CS-). This ensured that participants only consumed a small amount of food during conditioning (approximately 10 g), to prevent the development of sensory specific satiety, which could lower the reward value of the CS+. Unlike Experiment 1, self-reported hunger ratings, and cravings for sweet foods were equivalent before and after conditioning, suggesting this modification was successful and prevented the development of SSS. However, it is important to note that this single trial conditioning did not produce conditioned cravings as expected. Similarly, valence ratings of the CS+ failed to increase after conditioning. Overall, this means there was no evidence for appetitive conditioning at an explicit, psychological level.

In order to elucidate the reasons for this apparent lack of conditioning, exploratory analysis was conducted. Notably, during the conditioning procedure several participants commented to the researcher about the unpleasantness of the CS+, also reporting that marzipan was an unusual foodstuff to eat in isolation. Therefore, it was hypothesised that taste ratings of the marzipan object would be positively associated with degree of change on subjective measures of conditioning. In partial support of this, a weak positive relationship between taste ratings and CS+ valence change scores emerged. However, taste ratings were not associated with the degree of acquisition of other conditioned responses. These data partially confirm our expectations, suggesting the more participants enjoyed the taste of the marzipan object, the greater the change in perceived pleasantness from pre- to post-conditioning for this previously novel object.

It is unclear why participants in both Experiments 1 and 2 generally failed to respond to marzipan as a highly desirable substance. Blechert et al. (2016) utilised a very similar protocol, and reported that following conditioning, participants valued the marzipan-paired object as a highly craved, rewarding stimulus. However, as

Blechert's study was conducted in Austria, it is possible that cultural differences may have played a role. Food choice and preference is strongly influenced by cultural norms and values (Rozin and Vollmecke, 1986), and even practical factors such as food availability (Anna, 2001). A clear demonstration of this is the observation that insects are considered a delicacy in many Eastern countries, yet acceptance of insects as a food source is still very low among Western consumers (Hartmann & Siegrist, 2016).

Furthermore, food preferences and enjoyment are more complex than simply liking a taste or flavour. Taste ratings alone have proven problematic for predicting actual consumption behaviour (Lucas & Bellisle, 1987), whereas food appropriateness might be a better predictor of food acceptance and satisfaction, particularly in different contexts (Shutz, 1988; Cardello, Schutz, Snow & Lesher, 2000). A delicious ice cream may be highly coveted on a hot summer's afternoon, but receive little interest at breakfast in the cold winter months. The taste is equally delicious in both situations, but the appropriateness alters perceived enjoyment. Appropriateness can be driven by time of day, time of year and even be related to specific holidays and celebrations.

Marzipan is traditionally eaten at Christmas in Western Europe. In England it is most commonly served on a traditional Christmas fruit cake, rather than eaten in isolation. In contrast, across other parts of Europe, small marzipan figurines, in a variety of shapes and colours are a common indulgence; those marzipan figures share a much greater similarity to the shapes served in this experiment. Almli (2012) discovered that participants are more accepting of innovations in general, everyday meals than on traditional, festive foods. Therefore, the participants in our study may have been less accepting of marzipan when consumed in isolation, in an unusual colour and shape, particularly as the experiment took place in autumn, outside of the festive period – potentially interfering with the conditioning process. These assertions were supported via personal communications with Blechert who reported similar concerns about testing outside of the winter months. These factors highlight the importance of carefully selecting both highly pleasant and appropriate food items to serve as a CS+, particularly when the CS and US are presented in a compound form.

The pattern of data found on the implicit attention task was consistent with that of Experiment 1. At baseline, all distractor categories captured attention to a

similar degree on an EBA task. However, post-conditioning, performance accuracy was poorer when CS+, CS- and dessert images served as distractors, than for neutral distractors. It was hypothesised that the lack of differentiation in attentional capture by the CS+ and CS- in Experiment 1 allowed learning to spread from the CS+ to CS-, since the respective objects were presented simultaneously, through secondary conditioning. However, despite modifications to the conditioning procedure which should prevent this effect, the CS+ and CS- still captured attention to the same extent following conditioning in Experiment 2. In addition, both CS types also captured attention to the same extent as dessert images, which have been shown to be highly salient and desirable in previous experiments (Davidson, 2015).

At face value, this equivalence could be taken as an indication that these previously neutral, novel cues rapidly acquired the same degree of salience as cues with a long-standing reward history. However, the fact that desserts failed to capture attention to a greater extent than the neutral cues at baseline is problematic. If anything, it would be expected that desserts would be more salient than the other stimuli prior to consumption of a sweet food (de Pellegrino et al,. 2011). This may suggest that participants were lacking in motivation to eat prior to conditioning, despite moderate levels of hunger across the experiment, thus questioning whether the small increase in salience acquired across the experiment for non-neutral distractors can be truly attributed to reward learning.

These equivocal findings may be unsurprising given the degree of conflict in the literature regarding attentional bias for palatable, highly familiar food cues. Some evidence suggests that food-related attentional bias is greater for overweight and obese than lean individuals (Hendrikse et al., 2015), whilst others have found the opposite pattern (Nummenmaa et al., 2011). Similarly, there is debate about whether individual differences, such as restraint, play a role (Werthmann et al. 2013; Hollitt et al., 2010), whereas others have struggled to demonstrate a food-related attentional bias at all (Wilson & Wallis, 2013). Consequently, it seems that attentional capture by appetitive cues such as food is a complex phenomenon, and one not yet well understood. Further research is necessary to build a better understanding of the components underlying attentional capture for reward-related cues, and identifying how salience for certain cues may fluctuate depending on experience.

3.5 General Discussion

According to the incentive sensitization theory (Robinson & Berridge, 1993), reward-related cues (i.e., food and drugs of abuse) in the environment acquire incentive salience via appetitive conditioning. Through this reward learning, cues become "attention-grabbing", elicit cravings and approach motivated behaviours, and promote intake. A growing body of evidence supports the view that previously neutral stimuli can involuntarily capture attention after pairing with reward (Anderson, Laurent & Yantis, 2012). However, naturalistic paradigms for investigating these processes, which are quick and easy to implement, are lacking, and there is a scarcity of research in this area.

Building on the work of Blechert et al. (2016), the aim of Experiments 1 and 2 was to explore the utility of a novel, naturalistic conditioning procedure for imbuing an otherwise neutral object with incentive salience after a single learning trial, resulting in changes to implicit attention and explicit liking and cravings. Experiment 1 failed to find evidence that this single trial conditioning procedure produces robust differential appetitive conditioned responses on measure of cravings, and changes in perceived pleasantness for the CS+ were small. Contrary to expectations, both the CS+ and CS- were more distracting than neutral images post-conditioning on an EBA task. Several limitations of Experiment 1 were highlighted and addressed in Experiment 2, yet results were similar. In the latter study, there were no changes to explicit markers of conditioning, but again, the CS+, CS- (and also dessert) distractors all captured attention to a greater extent than neutral distractors after, but not before, learning took place.

The fact that explicit markers of conditioning were absent is problematic and may suggest that the appetitive US (marzipan) was insufficiently rewarding for successful differential appetitive conditioning to take place. Exploratory analysis in experiment 2 suggests that the more individuals enjoyed the taste of the CS+ object, the more likely they were to provide enhanced pleasantness ratings after conditioning. Consequently, it might be expected that a more rewarding CS+ would produce a robust increase in perceived pleasantness after conditioning. Research by Armel et al. (2009) has shown that the reward value associated with a particular US determines the degree of conditioning; abstract shapes paired with 'delicious' foodstuffs (e.g., ice-cream) attracted greater attention and received greater liking ratings than those paired with a pleasant but less highly-rated food (e.g., cane sugar).

Furthermore, Theeuwes and Belopolsky (2012) demonstrated that stimuli associated with a high monetary reward captured attention to a significantly greater degree than those linked to a low monetary reward. Perhaps a CS+ of higher reward value would show a greater ability to capture attention, over and above that of non-rewarded stimuli. Such variation highlights the importance of carefully selecting US of an appropriate value in studies of this nature. Maximising the reward value of the appetitive CS will be important for making more conclusive claims about the scope of single trial naturalistic conditioning.

The attentional data was also at odds with our original hypotheses. From an evolutionary perspective, the rapid and reliable detection of energy sources was essential for our ancestors. Overlooking a source of nutrition could be disastrous for health and survival. Thus, it is theorised that the human brain is geared to preferentially attend to cues signalling nutriment, which promotes food intake (Spence et al. 2015). This notion is supported in part by the attentional bias literature which shows that food captures attention to a greater extent than non-food images (e.g., Nummenmaa et al., 2011). Consequently, it was anticipated that after consumption of a novel edible object, its visual appearance alone would capture attention to a greater extent than visual presentation of a similarly novel plastic object that does not signal nutrient availability. Surprisingly, after conditioning a small, yet significant, impairment in the detection of not only the CS+, but also the CS- emerged, in comparison to neutral distractors.

This equivalence may be explicable when we consider that Nummenmaa et al. (2011) also reported that non-food items can capture attention to the same extent as food items when both are matched for visual appearance. Those authors argue that, through associative learning processes, a food's reward value becomes associated with its particular colour-shape combination, allowing for rapid detection. For example, a red sphere may become automatically associated with the sweet taste of a ripe apple. Whilst quick, it seems logical to assume that this detection mechanism could be prone to error. The costs of wasting energy and resources investigating a non-edible item would seemingly be less harmful than missing out on nutritious food sources. It may be then, that after just a single trial – particularly when very little is known about a novel food object, that attention is subsequently biased towards any item which may resemble or relate to the food in order to promote further exploration and potential discovery of other valuable food sources.

However, it is also possible that an alternative explanation can account for these attentional findings, independent of any learning taking place. The slight decrease in valence ratings for the neutral distractors across the experiment is of potential relevance, perhaps reflecting habituation to the experimental stimuli. Affective habituation can occur when individuals are repeatedly exposed to a liked stimulus, resulting in increased neutrality (Ferdenzi, Poncelet, Rouby & Bensafi, 2014). In addition, attention can habituate to irrelevant stimuli. Individuals who are pre-exposed to irrelevant stimuli find it easier to ignore in later attention tasks (Lorch & Horn, 1986). Consequently, neutral distractors may have been easier to ignore following exposure in the previous computer tasks, whereas other salient distractors may have been more resistant to habituation, perhaps due to their novelty or apparent relevance.

In addition, it is worth noting that all novel abstract shapes in our experiments were rated as slightly unpleasant prior to conditioning, based on their visual appearance. This slight negative bias seems to be in line with research on novelty and affect. Novel objects are typically perceived negatively upon first presentation. For example, Robinson and Elias (2005) found that novel faces with neutral facial expressions were rated as less likable than familiar faces with neutral facial expressions. The novel faces received ratings comparable to negative faces. The authors argue that liking increases with familiarity in a phenomenon known as the 'mere exposure effect'. Perhaps with repeated exposure, the effect of novelty of our food stimuli would be overcome, resulting in greater liking overall.

Finally, the conditioning procedure was relatively unusual and asked participants to pay direct attention to the different sensory characteristics of both object types, thus highlighting these features. Consequently, it could be argued that the conditioning procedure simply heightened the salience of the different objects, independent of their reward value. According to Theeuwes (2013), priming can exert a strong influence over the processing of a particular feature in a bottom-up fashion. Simply processing a particular feature, such as the colour red, causes stimuli which share that feature to be attended to first (Theeuwes & Van der Burg, 2013). Therefore, simple exposure to the novel objects, may have exerted a priming effect, ensuring that these stimuli subsequently received greater attention. Nonetheless, effects of priming on attention are typically very small and it is usually necessary to present primes repeatedly for effects to emerge. Thus, it seems unlikely that simple

priming effects can fully account for the present findings, and further exploration is warranted.

In conclusion, Experiments 1 and 2 failed to provide clear evidence of successful single-trial appetitive conditioning at an explicit level. The taste of marzipan appeared to be a weak US and may have minimised conditioning effects – despite the declaration of participants that they liked this food provided before the experiments. Both studies do, however, provide tentative evidence that a naturalistic single-trial appetitive conditioning procedure can modulate attentional processing of relevant cues. Novel food objects (CS+) capture attention more strongly than neutral distractors post-conditioning, although this was also true for novel plastic objects (CS-), and other similar cues. Whilst this generalised effect may represent an increased vigilance for potentially valuable food sources, more research is required to conclusively determine the processes underlying modulation of attention in a single trial.

Chapter 4

Single-trial appetitive conditioning: Unpicking the role of novelty, reward and salience.

4.1 Abstract

Previous research supports the utility of a novel, naturalistic appetitive conditioning paradigm for influencing motivational and attentional processed in humans, in just a single trial. However, Chapter 3 presented two experiments which failed to adequately replicate those findings when assessed by subjective reports and an implicit measure of attention (EBA task). Cravings and liking for an edible CS+ failed to increase after conditioning, perhaps reflecting the low reward value of the US (marzipan). Consequently, an alternative US was selected (chocolate) which was predicted to have higher reward value. For the present experiment, a neutral object became associated with the taste of this high value foodstuff (chocolate); participants were asked to consume this object, simulating a 'real' encounter with a new food. To determine how the novel and unusual nature of this conditioning paradigm might affect learning, participants were also exposed to a visually similar novel plastic object or a familiar household object, in a between-subjects design. Results from both subjective reports and an implicit attentional task confirm that a neutral stimulus paired with food can acquire incentive salience within a single trial. The novel edible object instantly became a highly liked and craved item after consumption, unlike novel or familiar inedible objects. Robust pre- to post-conditioning reduction in accuracy was apparent on an EBA task, whereby task-irrelevant images of an edible CS+ captured attention involuntarily, as did images of a visually similar object with no reward associations. This pattern of data may be suggestive of stimulus generalisation: learned salience rapidly spreads to stimuli with some similarity to the original CS, perhaps identifying an important mechanism which maximizes opportunities for reward-acquisition. This novel finding makes an important contribution to the current literature, particularly in today's obesogenic environment, and may have relevance for understanding hedonic overeating and obesity.

4.2 Introduction

Selecting an appropriate US is a significant challenge for appetitive conditioning research, leaving this a very understudied area (Herman, Zieglar, Birbaumer & Flor, 2000; Andreatta & Pauli, 2015). Chapter 3 presented data from two experiments designed to replicate a novel, naturalistic, single-trial appetitive conditioning paradigm, first described by Blechert et al. (2016). In this paradigm, the CS (sight of an object) and US (sweet taste) are combined to form a compound: novel geometric shapes were made from marzipan (CS+) or plastic (CS-) and either eaten or simply inspected, respectively. In traditional paradigms, where the CS and US are presented separately, any taste stimuli could theoretically be paired with a neutral stimulus. However, when the CS and US form a compound, particular challenges are raised when attempting to provide a sufficiently rewarding experience to support conditioning. In Chapter 3, despite the reported success of Blechert et al's methodology, a marzipan stimulus lacked sufficient reward value. Therefore, an alternative US was sought that could be easily moulded, easily coloured, hold its shape well, yet also be more palatable than marzipan.

As a worldwide popular sweet treat, chocolate seems like an obvious choice for a US. Chocolate is perhaps the most well-liked and craved food due to its particular sensory attributes (Rozin, Levine & Stoess, 1991). It has been said that 'chocolate inspires a passion normally reserved for things grander than food' (Roach, 1989, p.135) and there is some debate over whether chocolate is addictive in the same way as drugs of abuse (Bruinsma & Taren, 1999). However, it seems that the love of chocolate stems largely from its potent combination of fat and sugar, rather than any psychoactive properties (Yanovski, 2003). Therefore, a primary aim of this experiment was to examine whether stronger evidence of single trial appetitive conditioning would occur when chocolate served as a US, being more intrinsically rewarding and universally liked. To this end, the following experiments were conducted with abstract, coloured shapes manufactured from white chocolate.

Earlier, it was noted that performance in the emotional blink of attention task (EBA) suggested that both a novel CS+ and a novel CS- may be equally salient post-conditioning, as they captured attention to a similar degree. The original conditioning procedure was thus adapted so that the CS+ and CS- were presented separately, to remove the possibility of secondary conditioning, whereby the CS- could acquire incentive salience through its close physical and temporal association with the CS+.

However, the fact that there was still no decipherable difference between attentional capture by CS+ and CS- distractors suggests that other factors are at play.

Based on the lack of evidence for appetitive conditioning from self-report measures, it seems plausible that the saliency of the CS- could be driven by non-reward related processes. One possibility is that the observed effects derive from the mere novelty of these objects. The CS+ and CS- were deliberately novel, so as to have no prior association with food. However, novelty alone can bias attention. For example, attention is automatically orientated to novel over more familiar words in a well-known 'novelty pop-out effect' (Johnston, Hawley, Plewe, Elliott & DeWitt, 1990). Perhaps the equivalence of attentional capture by CS+ and CS- portraying distractors in the post-conditioning EBA reflects salience acquired merely through exposure to their novelty.

As discussed previously, it is well known that simple priming can enhance the salience of even the most basic visual stimuli, heightening their ability to attract attention (Theeuwes, 2013), which raises the possibility that the conditioning procedure itself acted as a prime. Simply holding the objects and being asked to pay attention to their sensory properties may have been sufficient to enhance their salience regardless of any reward associations.

Using a within-subjects differential procedure, where conditioned responses to an appetitive CS (CS+) are compared against those to a neutral CS paired with no reward (CS-), it is difficult to unpick the role of reward learning, stimulus novelty and simple priming since participants experience both object types. Thus, an alternative between-subjects design was devised, whereby three groups each handled only one object type: an edible, novel object made of chocolate; a visually similar, plastic object, or a familiar object. Although this design does not offer a true test of differential appetitive conditioning, it is the most convenient method for isolating each potential variable. If changes in attention truly represent appetitive conditioning, then only those in the CS+ group who have eaten the chocolate object should be specifically distracted by images of it in the post-conditioning EBA. However, if the effect is driven by priming, then each group should be distracted by images of the object they were assigned, but not those they have not handled. Finally, if the salience is enhanced by the object's novelty, images of the familiar object should fail to capture attention even after it has been primed through sensory exposure.

4.3 Methods

4.2.1 Participants

Seventy-five participants were recruited from the University of Liverpool and local area via online adverts and posters distributed across campus. Participants eligible for the School of Psychology Experimental Participation Requirement scheme were given course credit for participation. Those not eligible for credit were reimbursed for their time and effort with £15 in love2shop high street vouchers.

4.3.2 Design

A mixed design was employed with three experimental groups: an appetitive conditioning group; a neutral conditioning group, and a familiar exposure group (see Table 4.1). Attentional capture by various distractors, and self-report measures of conditioning were assessed before and after exposure/conditioning with one of three object types.

4.3.3 Exposure/conditioning

Each individual was assigned to one experimental group only. Participants were asked to provide measures of cravings and US-expectancy upon presentation of their assigned object; an appetitive CS (novel chocolate object), a neutral CS (novel plastic object) or a familiar stimulus (a red pencil; Table 4.1). The colour-shape combinations were as used in Experiment 3.2.

The appetitive CS was made from high quality Belgian chocolate and weighed approximately 10 g. The chocolate objects were created by a confectionary company which specialises in bespoke chocolate designs (Choc on Choc). It was not possible for the confectioners to colour dark or milk chocolate, so the objects were all made from white chocolate. The plastic-coated objects from Experiment 2 (Chapter 3) were re-used. The colour-shape assignment of the appetitive CS and neutral CS was counterbalanced across both groups (See Table 4.1) so that the chocolate object and the plastic object could be either yellow or orange.

Table 4.1 Object-assignment per condition with an example. Colour-shape assignment in the appetitive CS and neutral CS conditions was counterbalanced; half the participants in each condition received the orange object and the other half the yellow object.

Condition	Object Assignment	Example
Appetitive CS (n=26)	Novel abstract geometric shape made from chocolate.	
Neutral CS (n=25)	Novel abstract geometric shape made from plastic.	
Familiar stimulus (n=25)	A red coloured pencil.	

4.3.4 Self-report ratings

4.3.4.1 Craving and expectancies

Prior to handling the objects, participants were shown a colour photograph of their assigned object and asked to rate the degree of cravings and expectancies it elicited. Questions were adapted for this experiment from a study reported by Papachristou et al. (2013). Participants rated cravings and expectancies on 100 mm VAS scales presented in a counterbalanced order (Table 4.2).

Table 4.2 Wording of questions and VAS anchors for self-report craving and expectancy elicited by an appetitive CS, a neutral CS or a familiar stimulus.

Measure	Question	Anchors
Cravings	"When presented with this object, how strong is your craving for chocolate right now?"	"no craving at all" to "extremely strong craving".
US-Expectancy	"When presented with this object, how strongly do you now expect to be invited to eat chocolate?"	"certainly not" to "certainly".

4.3.4.2 Familiarity and valence

Participants completed a short computer task in which they were asked to rate the familiarity and valence of a series of images. Five images from each image category were randomly selected from the bank of images used in the EBA task. Images were rated for their subjective valence (pleasantness) and perceived familiarity using 100 mm VAS, with the anchors 'extremely unpleasant' to 'extremely pleasant' and 'extremely unfamiliar' to 'extremely familiar'. Images and question type were presented in a counterbalanced order. The ratings task was completed once before and once after conditioning.

4.3.5 EBA adaptations

The EBA task set up and procedure were consistent with Experiments 1 and 2 (Chapter Three), with the substitution of one of the distractor categories. Distractor images consisted of neutral images, images of both novel objects and images of the pencil, giving four distractor conditions: CS, Novel Stimulus, Familiar Stimulus and Neutral. It is important to note that as a between-subjects design was utilised, the attribution of each object depended on condition and counterbalancing (See Table 4.3). For those in the appetitive CS condition, one novel shape represented the edible CS (e.g., orange), whilst the other (e.g., yellow) was never handled by the participants. Thus, in this case, the orange object was coded as a CS distractor for

analysis, whilst the yellow object was coded as a novel stimulus. As colour-object assignment was counterbalanced this pairing was reversed for half the participants. The same pattern applied to those in the neutral CS condition; whichever plastic object participants handled was coded as a CS distractor, and the other as a novel stimulus. In the familiar stimulus condition, neither the yellow or orange novel objects were handled by participants, so had no acquired meaning. Consequently, the labels ‘CS’ and ‘Novel Stimulus’ have no real relevance. However, for the purposes of analysis, the yellow and orange objects were randomly assigned the label of ‘CS’ or ‘Novel Stimulus’.

Table 4.3 An example to show distractor types, and their associated meaning to participants after exposure, dependent on counterbalancing and condition. This table depicts an example when the orange object is assigned as the CS, but in half of cases, the reverse was true.

		Distractor Type			
		CS	**Novel Stimulus**	**Familiar Stimulus**	**Neutral**
		Orange Object 	*Yellow Object* 	*Pencil* 	*IAPS neutral images* 
Condition	**Appetitive CS**	CS (Chocolate)	Unhandled novel object	Pencil	Neutral
	Neutral CS	CS (Plastic)	Unhandled novel object	Pencil	Neutral
	Familiar Stimulus	Unhandled novel object	Unhandled novel object	Pencil	Neutral

4.3.6 Additional Measures

For consistency, the Dutch Eating Behaviour Questionnaire (DEBQ; Van Strien, Frijters, Bergers & DeFares, 1986) and Food Neophobia Scale (FNS; Pliner & Hobden, 1992) were measured again, in line with Chapter 3, to provide descriptive information about the sample. In addition, the role of impulsivity/reward sensitivity in appetitive conditioning is unclear with some studies showing no associations (Papachristou et al., 2013), but others suggesting a possible association between impulsivity and sensitivity to appetitive conditioning, at least on certain measures (Wardle, Lopez-Gamundi & Flagel, 2018). Consequently, the BAS-RR subscale of the BIS/BAS (Carver & White, 1994) and the 8-item Brief Sensation Seeking Scale (BSSS-8; Hoyle et al, 2002) were used to provide descriptive information about the sample and to ensure conditions were well matched on these variables. [See Chapter 2 for a full description of all self-report scales].

4.4 Procedure

Participants first completed the screening as described in the general methods (chapter 2). Eligible participants who expressed a liking for white chocolate (i.e., scored 6 or above on a 9-point scale) and declared no food allergies were first assigned to the appetitive CS group, until the full quota was filled for that condition. Participants who declared a food allergy or a dislike for white chocolate, yet met all other criteria were randomly assigned to either the neutral CS condition or familiar exposure condition. Participants were tested between 11:00 and 17:00, and asked to fast for a minimum of two hours prior to the experiment. Upon arrival at the lab, participants provided informed consent and rated their current appetite followed immediately by the EBA task. A subset of images from each image category in the EBA task were then rated for pleasantness and familiarity on VAS scales in a separate computer task.

Participants were presented with their assigned object and asked to rate cravings and expectancies for chocolate Both baseline ratings were provided based on sight alone, before participants handled the objects. Participants were only asked to rate the object associated with their condition to minimise any effects that exposure to the other objects may have had on the results. Participants were then asked to touch, handle, smell and, in the appetitive CS group, to taste their assigned

object for two minutes and then rate its sensory characteristics. Following this exposure task, participants again completed appetite ratings, followed by the final, post-conditioning EBA session. Measures of craving and expectancy in response to the respective object were repeated. Participants also completed the battery of questionnaires designed to measure reward sensitivity, food neophobia and eating related attitudes. Height and weight were measured with scales and a stadiometer. Finally, participants were given a full verbal and written debrief and thanked for their time.

4.5 Results

4.5.1 Participant characteristics

In total, 75 participants completed the full experiment. One participant displayed particularly poor performance on the EBA task with less than 25% accuracy in total (below chance level). A further participant in the appetitive CS condition strongly disliked the chocolate object and refused to eat it after a small bite. Consequently, these participants' data were removed from all analysis, and results are presented for the remaining 73 participants. Due to technical issues, computerized VAS ratings for three participants failed to record. These instances were coded as missing data in SPSS, and the remaining data for these participants were kept in the analysis.

Each experimental condition was well matched on most baseline characteristics (see Table 4.4). Those in the familiar stimulus condition scored slightly lower for restrained eating than those in the neutral CS condition, $p = .003$. However, overall levels of restraint were low in both groups, and as neither were given any food during the study I do not believe that this small difference introduces significant confounds.

In terms of personality variables, four outliers were identified but their exclusion did not alter the pattern of results so they remain in the full analysis. Levels of food neophobia were similar to that reported in a comparable sample of European university students (Fenko, Leufkens & van Hoof, 2015; mean ± SD: 29.39 ± 10.07), with scores ranging from 10 – 59. Eating attitudes were slightly elevated compared to mean scores of a sample of Dutch undergraduates on DEBQ subscales for restraint, emotional eating and external eating (2.47 ± 0.88, 2.48 ± 0.71 and 3.13 ± 0.51, respectively; van Strien, Herman & Anschutz, 2011). The BAS-RR

scores were comparable to those of moderate scorers in a non-clinical sample (16.83 ± 2.26; Alloy, et al. 2006). The Brief-SSS: Total was also similar to that of a UK University based convenience sample (3.01 ± .59; Eachus, 2004)

All participants in the appetitive CS and neutral CS conditions demonstrated an awareness of the CS-US contingencies and could accurately recall the colour-shape combinations associated with chocolate or plastic, respectively. Three participants identified the experimental aims with some accuracy, by suggesting that the experiment was exploring how associating objects with edibility influences task performance on the EBA task. Two further participants mentioned associations between an object and chocolate changing attitudes/desire. Removal of these participants did not change the overall pattern of results and so to maintain power, all participants were retained in the full analysis.

Table 4.4 Participant characteristics for each experimental condition (M ± *SD*).

	Total	**Appetitive CS**	**Neutral CS**	**Familiar Stimulus**
N	73	24	25	24
Gender	50F, 23M	17F, 7M	17F, 8M	16F, 8M
Age	21.23 (*4.89*)	20.71 (*5.05*)	20.88 (*3.79*)	22.13 (*5.76*)
BMI	23.33 (*3.78*)	22.29 (*2.56*)	23.19 (*3.61*)	24.54 (*4.67*)
FNS	26.96 (*11.74*)	28.38 (*10.87*)	26.60 (*12.00*)	25.92 (*12.62*)
DEBQ				
Restraint	2.62 (*.84*)	2.70 (*.76*)	2.87 (*.90*)	2.27 (*.77*)
Emotional	2.86 (*.90*)	2.78 (*.83*)	3.12 (*.87*)	2.69 (*.96*)
External	3.52 (*.56*)	3.44 (*.58*)	3.61 (*.54*)	3.51 (*.58*)
BIS/BAS				
BAS-RR	17.14 (*2.44*)	17.00 (*1.87*)	17.76 (*2.11*)	16.63 (*3.15*)
Brief-SSSS	3.39 (*.72*)	3.53 (*.57*)	3.35 (*.76*)	3.28 (*.82*)

Mean (SD) for different measures. BMI = body mass index, DEBQ = Dutch Eating Behaviour Questionnaire; FNS = Food Neophobia Scale; BIS/BAS = Behavioral Inhibition System (BIS) and the Behavioral Activation System (BAS); BAS-RR = Reward Responsivity; Brief-SSS = Brief Sensation Seeking Scale. As the DEBQ has a distinct three-factor structure a total score was not computed.

4.5.2 Appetite ratings

A mixed ANOVA revealed a significant main effect of time (pre- or post-exposure) on appetite ratings, $F(1, 70) = 19.35$, $p < .001$, $\eta_p^2 = .22$, but the main effect of condition, and the condition x time interaction failed to reach significance (ps>.05) (See Table 4.5 for descriptives). Overall, average appetite ratings increased from pre-conditioning (57.32 ± 18.09), to post-conditioning (63.10 ± 18.09), regardless of condition ($p < .001$).

Table 4.5 Average appetite ratings for each experimental condition pre- and post-exposure.

	Stimulus Type		
	Appetitive CS	Neutral CS	Familiar Stimulus
Time point	Mean (*SD*)	Mean (*SD*)	Mean (*SD*)
Pre-conditioning	59.40 (*13.28*)	50.71 (*20.03*)	61.84 (*20.01*)
Post-conditioning	62.77 (*12.18*)	59.14 (*20.38*)	67.39 (*20.32*)

4.5.3 Subjective measures of appetitive conditioning

4.5.3.1 Appetitive CS Taste Ratings

Participants in the appetitive CS condition generally rated the taste of the chocolate object as highly pleasant (78.79 ± 10.30; Min = 39, Max = 96), and ate all the chocolate provided (9.18 g ± 0.71).

4.5.3.2 Conditioned Expectancies

A 3×2 mixed ANOVA revealed a significant main effect of time (pre-, post-conditioning) and condition (appetitive CS, neutral CS and familiar stimulus), as well as a significant time x condition interaction on expectancies for chocolate, $F(2, 70) = 26.02$, $p < .001$, $\eta_p^2 = .43$.

Exploring the simple effect of time within each condition revealed that expectancies for chocolate remained consistent at both time points for those exposed to a neutral CS, $t(24) = 2.93$, $p = .574$, $d = .46$. The change in expectancies for those exposed to a familiar stimulus did not survive a Bonferroni correction, $t(23) = 2.27$, p

=.033, d = .11. For those who ate the appetitive CS, expectancies for chocolate increased significantly from pre- to post-conditioning, $t(23) = 7.32$, $p < .001$, d = 1.49, suggesting, as anticipated, that appetitive conditioning occurred in this group only (see Figure 4.1).

One-way ANOVAs were used to explore the simple effect of condition at each time point, followed by *post hoc* tests with Bonferroni corrections applied. Prior to conditioning/exposure, expectancies for chocolate elicited by the respective object differed slightly between groups, $F(2, 70) = 4.60$, $p = .013$, $\eta_p^2 = .12$. Compared to the familiar stimulus the appetitive CS and neutral CS elicited slightly stronger expectancies for chocolate at baseline (ps ≤.046). This was perhaps driven by uncertainty about the properties of novel, compared to familiar stimuli. Post-conditioning, there was a clear difference between conditions on expectancies for chocolate, $F(2, 70) = 24.99$, $p < .001$, $\eta_p^2 = .42$. Whilst the familiar stimulus and neutral CS elicited similar expectancies ($p = 1.00$), the appetitive CS elicited significantly greater expectancies for chocolate than both non-edible objects (ps < .001).

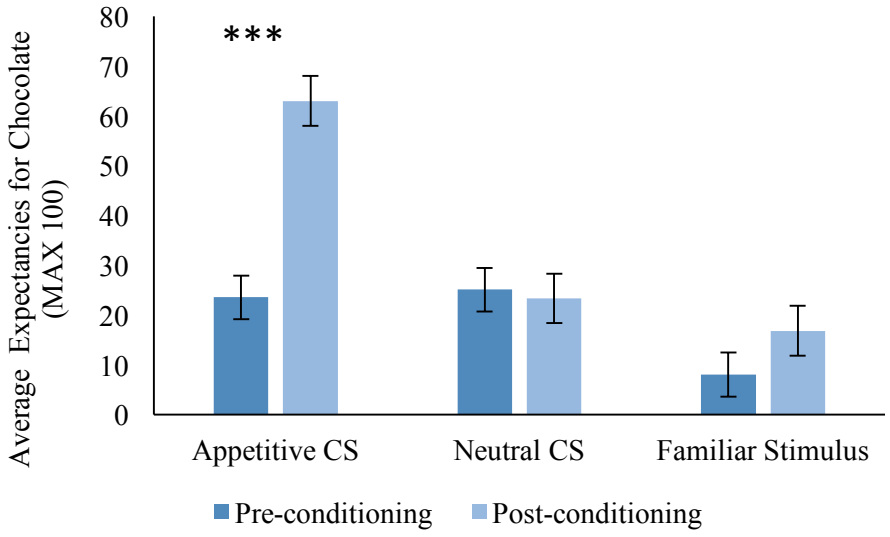


Figure 4.1 Average expectancies for chocolate rated on 100 mm VAS scales pre- and post-conditioning for each condition, depending on whether participants were exposed to an appetitive CS, a neutral CS or a familiar stimulus.

4.5.3.3 Conditioned Cravings

A 3×2 mixed ANOVA revealed a significant main effect of stimulus condition (familiar stimulus, appetitive CS or neutral CS), a significant main effect of time

(pre-, post-conditioning), and of particular interest, a significant time × condition interaction on conditioned cravings for chocolate, $F(2, 70) = 13.43$, $p < .001$, $\eta_p^2 = .28$.

Paired t-tests were conducted to explore the simple effect of time within each condition. Clear evidence of conditioned cravings emerged in the appetitive CS condition, with a large increase in conditioned cravings from pre- to post-conditioning, $t(23) = 5.42$, $p < .001$, $d = 1.11$. As predicted, cravings elicited by the novel CS were similar before and after conditioning, $t(24) = 1.50$, $p = .148$, $d = .03$. Contrary to expectations, there was a small, yet significant increase in cravings elicited by the pencil in the familiar stimulus condition from before to after handling, $t(23) = 2.93$, $p = .007$, $d = .60$ (See Figure 4.2).

One way ANOVAs were used to explore the simple effect of condition at each time point, followed by *post hoc* tests with Bonferroni corrections applied. Prior to conditioning/exposure, levels of cravings elicited by the respective object did not differ between groups, $F(2, 70) = 1.47$, $p = .238$, $\eta_p^2 = .04$. However, differences emerged in the post-conditioning measures, $F(2, 70) = 12.53$, $p < .001$, $\eta_p^2 = .26$. Conditioned cravings were significantly larger for the appetitive CS condition than the novel CS condition, $p<.001$, or the familiar stimulus condition, $ps<.001$.

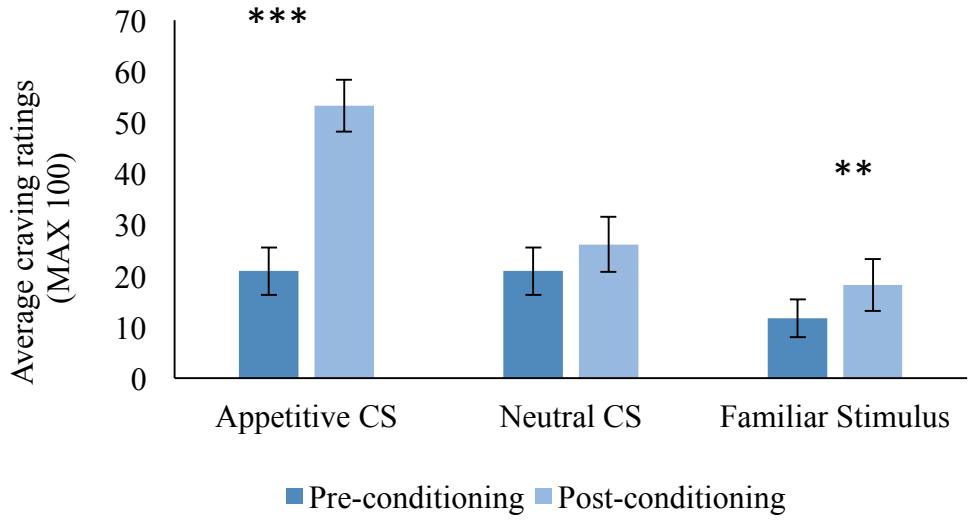


Figure 4.2 Average craving ratings before and after conditioning/exposure to an appetitive CS (chocolate object), neutral CS (plastic object) or a familiar stimulus (pencil) dependent on condition. Bars represent standard error. *$p<.05$, **$p<.01$ ***$p<.001$.

4.5.4 EBA

4.5.4.1 Percentage Accuracy

A 3×2×2 Mixed ANOVA was conducted on target identification accuracy on EBA trials dependent on distractor type (% correct responses, where a lower score indicates greater attention capture). This revealed significant main effects of time, $F(1, 70) = 7.27$, $p = .009$, $\eta_p^2 = .094$, and distractor type, $F(2.58, 180.23) = 83.95$, $p < .001$, $\eta_p^2 = .55$, on percentage of correct responses on the EBA task. Additionally, there was a condition × distractor type, $F(5.15, 180.23) = 4.34$, $p < .001$, $\eta_p^2 = .110$, and a time × distractor type interaction, $F(3, 210) = 4.34$, $p = .001$, $\eta_p^2 = .110$. Of particular interest was the significant condition × time × distractor type interaction, $F(6, 210) = 2.15$, $p = .049$, $\eta_p^2 = .06$.

In line with our hypotheses, of major interest was whether conditioning or exposure to the different objects influenced the change in accuracy for relevant distractors from pre- to post-conditioning/exposure. Consequently, data were split by condition and the time × distractor type interaction was explored. Within the familiar stimulus condition, the time × distractor type interaction failed to reach significance ($p > .05$), but there was a main effect of distractor type, $F(2.13, 48.95) = 43.14$ $p < .001$, $\eta_p^2 = .652$. Images of the familiar stimulus (pencil) captured attention to a greater extent than all other distractors at both time points, Ps<.001 (see figure 4.3C).

By contrast the time × distractor type interaction was significant for both the appetitive CS condition, $F(3, 69) = 4.02$, $p = .011$, $\eta_p^2 = .149$., and the novel CS condition, $F(3, 72) = 4.34$, $p = .016$, $\eta_p^2 = .133$. Post-hoc paired t-tests revealed that performance on the EBA task remained the same from pre- to post-conditioning for all distractor types in the neutral CS condition (ps ≥ .057) suggesting that accuracy for these distractors did not change as a result of exposure to a novel plastic object (see figure 4.3B). However, when focusing on the appetitive CS condition, there was a clear decrease in accuracy from pre- to post-conditioning for the CS (chocolate object) ($p = .001$) and novel stimulus which was never handled ($p = .002$), suggestive of an increase in salience attributable to naturalistic conditioning with an edible object (See figure 4.3A)

For completeness, the simple effect of distractor type was also explored at both time points within each condition. Pre-conditioning, the CS and novel stimuli captured attention to the same degree as neutral distractors across all conditions but

unexpectedly, the familiar stimulus (pencil) was significantly more distracting than all other distractor types (*p*s < .001).

Post-conditioning, the pattern was slightly different for the appetitive and neutral CS conditions. In the neutral CS condition, accuracy was higher for neutral distractor trials than all other distractor types (*p*s ≤ .019). Within the appetitive CS condition neutral distractors were least distracting overall (*p*s ≥ .025) and the familiar stimulus captured attention to a greater extent than CS (chocolate) and novel unseen distractors (*p*s ≤ .013).

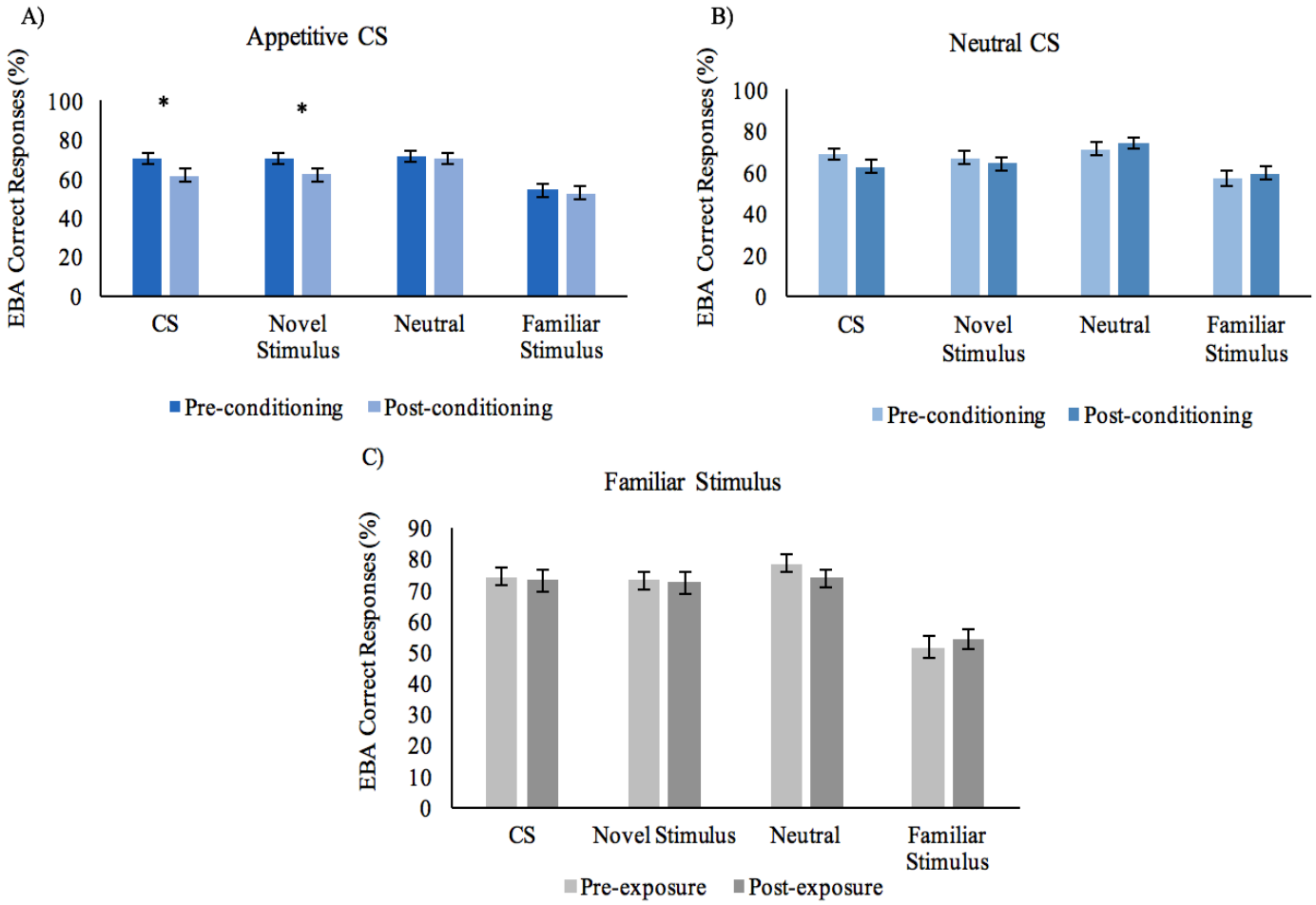


Figure 4.3 Average target detection accuracy on an EBA task before and after conditioning/exposure to A) an appetitive CS (edible white chocolate object) , B) a neutral CS (inedible plastic object) or C) a familiar stimulus (red pencil). Bars represent standard error. * *p*s < .05.

4.5.4.2 Reaction times

Reaction times on the EBA task before exposure/conditioning (268.01 ms ± 106.92) were significantly slower than reaction times after (241.36 ms ± 103.81), $F(1, 70) = 30.42$, $p = .001$, $\eta_p^2 = .30$. No other main effects or interactions reached significance ($ps \geq .338$)

4.5.5 Computerized VAS ratings

4.5.5.1 Familiarity Ratings

Mixed ANOVA revealed no main effects or interactions between condition, time point or image type ($Ps > .05$) on ratings of familiarity. This suggests, contrary to our expectations, that all images were equally familiar to participants regardless of their exposure condition and the time of measurement (see Table D1, Appendix D).

4.5.5.2 Valence Ratings

Mixed ANOVA revealed no main effects or interactions between condition, time point or image type (Ps>.05). This suggests that all images were rated as equally pleasant at both time points, regardless of experimental condition (see Table D2, Appendix D). Contrary to expectations, participants in the Appetitive CS condition did not find the photographs of the CS object (made from chocolate) more pleasant after conditioning.

4.5.6 Associations with individual differences

Correlations were conducted between conditioned responses (difference scores; Post-Pre) for self-report measures (craving and expectancy) and difference scores (Post - Pre) for attentional capture on EBA task) and self-report measures of individual differences (e.g., eating attitudes, reward sensitivity) within the appetitive CS condition. (See Table D3, Appendix D for correlation matrix). After applying statistical corrections, no correlations reached significance.

4.6 Discussion

The main aim of this experiment was to determine whether a previously neutral cue would acquire incentive salience after just a single learning trial when CS reward value was maximised by selection of a highly palatable and appropriate US, in this case white chocolate, substituting for the less rewarding marzipan used previously. As the naturalistic conditioning paradigm is relatively unusual for participants, a further aim was to explore whether other features associated with this process, such as exposure to a novel object in general, or simply focusing on sensory characteristics of any object could account for modified salience.

For participants given an appetitive CS, self-report ratings showed clear evidence of appetitive conditioning taking place. In particular, a novel edible object was transformed into a craved cue after eating, and participants came to strongly expect this object to predict chocolate availability in the future. Participants given a visually similar plastic object, failed to show these changes – in line with our hypotheses. Unexpectedly, cravings for chocolate increased slightly after participants handled a familiar object (pencil). However, compared to the change observed with the chocolate CS, this was a minimal effect which most likely reflects simple priming due to the wording of the question. Overall, the change in conditioned cravings from before object exposure to immediately after was most marked, and significantly larger for the appetitive CS.

Changes in explicit measures of conditioning were matched by alterations to implicit measures. As predicted, for those in the appetitive CS condition, there was a significant drop in EBA accuracy from pre- to post-conditioning on trials where a CS distractor was presented, suggesting that just one learning episode with an edible 3D chocolate shape imbued an otherwise neutral, novel object with incentive salience, rendering it capable of automatically capturing attention even when presented under conditions of limited awareness. This is consistent with the view that motivationally relevant stimuli are given pre-attentive prioritization allowing them to ‘win’ over competing stimuli and to automatically enter awareness (Most & Junge, 2008; Most & Wang, 2011). Taken together with the self-report data, this effect provides more convincing evidence for appetitive conditioning in a single trial. For those participants given a novel or familiar stimulus with no reward associations, distractors representing these objects caused no such reduction in correct EBA

responding, strengthening support for the proposal that the reduction in accuracy in the CS condition reflects a reward-related mechanism.

What is particularly interesting is the fact these motivational and attentional changes occurred so rapidly. From an evolutionary perspective, such fast learning may have proven an adaptive mechanism to maximise opportunity for acquiring sufficient nutriment, ensuring no potential food sources were overlooked. However, in today's obesogenic environment, where highly calorific foods are abundantly available, this facility may prove counterproductive, as a near continuous stream of cues associated with sweet, energetic foods may enter awareness. Even from early childhood humans are likely to have acquired innumerable reward associations with food-related cues. Thus, therapeutic strategies aimed at preventing such associations are unlikely to be effective, as they would rely on complete avoidance of new stimulus pairings. Instead, it may be more fruitful to focus on improving methods of altering conditioned responses, such as attentional bias modification (e.g., Smith, Treffiletti, Bailey & Moustafa, 2018).

It is also important to note that, contrary to expectations, a visually similar novel object captured attention to a greater extent after consumption of the appetitive CS despite having no direct associations with reward, and having never been seen or handled in 'real life'. Whilst this change may seem at odds with our above conclusions, this phenomenon is worthy of further consideration. Under some circumstances, it seems that conditioned responses are able to spread via a mechanism called stimulus generalisation; an otherwise neutral stimulus can elicit conditioned responses if it shares some similarity with a conditioned stimulus. Generalisation is typically seen as an adaptive process (Dunsmoor, Mitroff & LaBar, 2009): it would be time-consuming and unrealistic to require an encounter with each individual variation of a stimulus for learning to be complete, and subsequent recognition to occur. The ability to generalise between similar stimuli allows organisms to respond rapidly and appropriately to a wide range of categorically-related stimuli, irrespective of whether a specific variant has been encountered before.

Despite wide support for stimulus generalisation using aversive stimuli (Lissek et al., 2008), to date there has been no attempt to demonstrate this phenomena in human subjects using appetitive stimuli. The apparent spread of learned attentional prioritisation from an appetitive CS to a visually similar novel

object in the present study may be tentatively interpreted as support for this phenomenon in humans and warrants further investigation. Unfortunately, it is not possible to determine whether generalisation would have also been evident for subjective conditioned responses. In order to minimise exposure to the different classes of object, participants were only asked to provide subjective self-reports in response to their assigned object, before and after they were given this object to handle Further exploration of appetitive conditioning and stimulus generalisation across implicit and explicit measures may prove a valuable area for future research.

Some degree of caution is, however, warranted when interpreting these results – specifically in relation to the impact of novelty *per se* on attentional measures. Although no significant reduction in pre- to post-conditioning accuracy was observed for those exposed to a Neutral CS (novel plastic object), when analysis focused solely on EBA performance post-conditioning/exposure, both a neutral CS (plastic) and a similar novel object captured attention to a greater extent than neutral items overall. As neither item had any reward associations, this perhaps suggests a small effect of novelty in general. Evidence from fMRI research highlights that novelty can act as a signal to motivate further exploration of a stimulus in search of potential rewards (Krebs, Schott, Schütze & Düzel, 2009). Therefore, these data may allude to a possible synergistic role of novelty and reward to promote exploration and enhance learning about previously unknown stimuli. This sort of mechanism could prove potentially useful for maximising the probability of obtaining reward or avoiding punishment when properties of novel objects are uncertain. However, it is not possible to draw any firm conclusions or make further inferences from the present data, particularly after just one learning trial. Future research may determine whether any initial effects attributed to novelty diminish over multiple trials as stimuli become more familiar to participants.

In addition, whilst performance on the EBA task appeared to be unaffected by exposure to the familiar stimulus, overall accuracy on trials where this stimulus – a red pencil – was present was particularly low, at both time points and across all conditions. Poor performance on the EBA is typically attributed to the affective properties of distractor images (Most et al., 2005). Such significant attentional capture might be expected for highly arousing stimuli, such as graphic images of violence (Most & Junge, 2008) or erotic images (Most et al., 2007) but not for a plain red pencil. Familiarity ratings confirmed that this object was not unfamiliar to

participants, and valence ratings suggest it was relatively neutral. Although arousal ratings were not collected, it seems unlikely that this object would have been particularly arousing. Thus, it seems a non-affective explanation for this unexpected salience would be more parsimonious.

In support of an alternative account, there is evidence that certain properties of a stimulus may interact with a viewer's current goal set, causing an otherwise neutral distractor to capture attention (Folk, Remington & Johnston, 1992). This phenomenon is known as *contingent attentional capture* and describes the finding that neutral stimuli which share some similarity to a target are more salient. For example, when a target is defined in a particular colour, distractors of the same colour automatically capture attention, whereas distractors which share no features with the target are easily ignored (Folk, Leber & Egeth, 2002).

Ariga and Yokosawa (2008) developed this model further by demonstrating that even if a distractor differs from a target on a physical level, it can still capture attention if it shares a property of the target on an abstract level. In their experiment, the target was defined as a green Japanese *kanji* character. Distractors whose meaning was congruent with the target (a white character which meant 'green' in Japanese) impaired target detection, whilst incongruent distractors failed to capture attention. In a further demonstration, target detection (e.g., tennis ball) was impaired by conceptually relevant distractor images (e.g., tennis court) (Sulman & Sanocki, 2011).

Whilst unintentional, and at odds with our experimental aims, it seems plausible that contingent attentional capture may account for the unexpected capture by images of pencils. The photographs of the pencils were taken at various angles on different neutral backgrounds, with the pencils shown at various angles in different orientations. For example, the pencil may have been displayed horizontally on a table or upright in a pencil pot, offset at a 45-degree angle. As participants were searching for a rotated landscape image on each trial, the physical properties of the pencil – with its linearity and sharpened point providing directional information – may have added confusion over whether the pencil was a rotated target. Although this hypothesis was not directly tested, verbal reports by a small number of participants during the debrief seem to support this assertion. Consequently, further research would be needed, using a more appropriate familiar stimulus, to determine

conclusively whether the handling of any object, regardless of its affective properties or novelty would render it more salient and thus more distracting.

The present study was subject to a number of further limitations. Firstly, the decision to use a between-subjects design meant that, although the separate effects of reward learning, novelty and mere exposure could be explored, it was *not* possible to demonstrate true differential conditioning and thus the possibility that those in the appetitive CS condition would not have responded in a similar fashion to the neutral CS cannot be ruled out.

Furthermore, self-report familiarity ratings failed to support a difference in perceived familiarity between the novel objects and the familiar pencil. Additionally, valence ratings revealed no differences between image categories regardless of experimental condition; all images were rated as neutral at both time points. It is surprising that no increase in valence was observed for the appetitive CS after consumption, considering the clear acquisition of conditioned cravings and expectancies.

However, the computer rating tasks themselves may have been problematic. Participants were asked to rate colour photographs randomly selected from those used in the EBA task. These images were thus a mixture of neutral photographs from the IAPS database, a selection of photographs of novel 3D-shapes (yellow and orange), and a red pencil. Each object type was photographed against similar everyday household backgrounds for consistency. Task instructions simply asked participants to rate the image for how pleasant and how familiar they found it. Therefore, participants may have considered the background, as well as foreground object, potentially affecting responses, particularly when rating the objects associated with chocolate against backgrounds not typically associated with food and edibility. Addressing these limitations may prove important for better understanding of the synergistic effects of novelty and reward on attentional capture and appetitive conditioning processes in general.

To conclude, we have successfully demonstrated single-trial appetitive conditioning, employing a novel paradigm to promote the learned association of a unique, previously unseen, novel stimulus and food reward. Furthermore, it was determined that this object-reward association is reflected in enhanced involuntary attentional capture by briefly presented, task-irrelevant images of a food CS+, signifying the acquisition of incentive salience by a novel stimulus. Moreover,

stimulus generalisation was also observed, whereby visual stimuli sharing some features of the CS+ also acquire salience and preferential attentional processing. These combined effects indicate the operation of automatic processes that maximise the detection of motivationally-salient, potentially rewarding stimuli, by allocating attentional resources even in the absence of a conscious search – exemplifying not only the establishment of precise, specifically-experienced contingencies but also category learning.

Chapter 5

Subjective and attentional markers of appetitive conditioning across multiple trials.

5.1 Abstract

Previously neutral cues can acquire incentive salience through Pavlovian conditioning. Recent research has raised the possibility of such learning occurring in just a single-trial when a naturalistic paradigm is employed, yet findings across Chapter 3 and 4 have been inconsistent. Currently, little is known about the time course of Pavlovian conditioning and the optimum trial number. A core aim of the present experiment was to measure subjective and attentional markers of naturalistic conditioning across multiple trials. Using a within-subjects design, participants (n=37) underwent four naturalistic conditioning trials in which the appearance of a neutral object was conditioned to chocolate taste (CS+) or non-reward (CS-), by asking participants to eat the CS+ object (made from white chocolate) or handle the CS- object (made from plastic). Clear evidence of differential appetitive conditioning was evident in terms of subjective conditioned responses. Cravings, US-expectancies and liking were learned rapidly, with the greatest change occurring after the first conditioning trial. No evidence emerged to suggest that conditioning modulated attentional processing. This experiment provides further support for the strength of naturalistic single-trial conditioning. The lack of evidence for the modulation of attention by naturalistic appetitive conditioning shows that more research is needed to understand how value-driven attentional capture may shape food-related attentional biases and human eating behaviour. The findings are discussed in terms of theoretical and methodological implications.

5.2 Introduction

Pavlovian conditioning has been implicated in motivational (i.e., cravings, desire, liking) and attentional processing of reward-paired cues. Value-driven attentional capture is considered a central determinant of aberrant attentional processing of reward-related stimuli (Anderson, 2016). Therefore, understanding reward learning may lead to a fuller account of the mechanisms underlying attentional capture by motivationally salient stimuli, such as high calorie junk foods. However, it is currently unclear how fast this process is, and how many trials are necessary for successful learning to take place.

Notably, Blechert et al. (2016) demonstrated the utility of a novel, naturalistic single-trial conditioning procedure, which successfully modulated implicit early and late event related potentials (ERPs), and explicit self-report measures of craving elicited by a novel, palatable food CS+ in just a single-conditioning trial. Utilising this novel conditioning paradigm, experiments 1 and 2 (Chapter 3) failed to find evidence for appetitive conditioning in a single trial based on explicit, self-report measures. However, when more desirable chocolate served as an appetitive US, in place of marzipan (as employed by Blechert et al., 2016), evidence of conditioned cravings, expectancies and evaluative changes were evident for a CS+, but not a CS- or familiar neutral stimuli.

Our findings suggest that, when the value of the US is sufficiently high, appetitive conditioning can occur rapidly. However, when assessing attentional capture via an emotional blink of attention task (EBA), such rapid differential appetitive conditioning appears difficult to demonstrate, questioning the number of CS-US pairings required for successful conditioning to take place. Overall, our results suggest that following a novel, naturalistic conditioning paradigm, both edible objects (CS+) and inedible objects (CS-) acquire salience and subsequently capture attention more than neutral images when presented as task irrelevant distractors in an EBA task. This equivalence runs counter to our original hypothesis that attention would be modulated specifically by reward learning.

According to Esber and Haselgrove (2011), both the physical properties of a stimulus, and the significance it acquires through experience, determine its overall salience. In turn, experience may influence attentional processing in a variety of ways. Most research has focused on the role of *reward expectancy* on learning and attention, or the level of reward associated with a particular cue. For example, studies

have reliably shown that attention is greater for cues paired with reward compared to no reward, or for high- versus low-reward associated cues (Della Libera & Chelazzi, 2009).

However, *reward uncertainty* regarding the CS-US relationship also appears to be important. This term refers to the probability of reward, or the prediction error. It could be argued that it is more adaptive to guide attention towards cues that reliably predict reward, such as fruits that have been safely ingested on many occasions, while ignoring those cues that are less certain indicators. This notion is summarised by a model proposed by Mackintosh (1975), who argued that cues highly predictive of reward are attended to more than cues with a low probability of reward (uncertain cues). According to Le Pelley et al. (2016), there is a wide body of evidence in support of this model. For example, using eye-tracking technology, Le Pelley, Beesley & Griffiths (2011) found that dwell time was significantly longer for cues that were highly predictive of an outcome than for non-predictive cues.

Conversely, focusing attention solely on predictive cues could limit learning and experiences. If all uncertain cues are ignored, new opportunities may be missed. Therefore, under certain circumstances, focusing attention on uncertain cues could be more adaptive. This notion is exemplified by the Pearce and Hall model (1980), which has also garnered broad empirical support. This uncertainty hypothesis posits that if a stimulus-outcome association is uncertain, then this relationship must be continuously monitored and thus warrants greater attentional processing (De Tommaso, Mastropasqua & Turatto, 2018).

The pattern of data outlined in Chapter 3 suggests that after just one learning trial all newly learned novel cues are equally salient. It seems plausible that a single trial is insufficient to make accurate predictions about the strength of an association between the CS and US, so that a degree of uncertainty will likely remain. In this case, when available information about predictiveness is limited, uncertainty may guide attention, such that both the CS+ and CS- attract attention in order to maximise opportunities for learning about these novel objects and their reward associations. However, it is unclear how attentional processing will change over successive trials, as predictiveness increases.

Furthermore, research in primates highlights that both reward and novelty are important determinants of attention, and that their influence changes with experience (Ghazizadeh, Griggs & Hikosaka, 2016) (see Figure 5.1). When animals were

exposed to different cues, the experimenters found that all novel objects were initially salient, highlighting that novelty can affect attention independently of reward. As discussed above, this is likely due to uncertainty about the outcomes associated with a novel stimulus. It seems adaptive to allocate greater attentional resources to objects of which little is known. Any novel object could theoretically imply significant rewards, but also be hazardous or unpleasant. Therefore, attention to these objects is warranted to allow learning to take place. However, with repeated experience, the primates became increasingly certain about the properties of the once novel stimuli: novel objects paired with reward became more salient and increasingly captured attention, whereas mere exposure to novel objects in the absence of reward learning simply increased their familiarity. As familiarity increased, the novel but neutral objects became less salient and were thus ignored. Consequently, the following study aimed to assess appetitive conditioning over multiple trials to explore whether a similar pattern emerges for novel cues paired with a rewarding or neutral outcome.

As mentioned previously, many studies use a large number of learning trials. However, these designs are often impractical and likely to induce significant fatigue and boredom. Furthermore, the growing evidence supporting single trial conditioning would also suggest that these complex and lengthy test procedures are not a necessary requirement for successful appetitive conditioning in humans.

Papachristou et al. (2013) were able to utilise a simple differential appetitive conditioning paradigm, with chocolate as a US, which resulted in strong appetitive conditioned responding within just four learning trials. Ingestion of chocolate (CS+) and no reward (CS-) was paired with one of two coloured trays. On each trial, participants were asked to rate their cravings for chocolate, their expectancies regarding chocolate availability, and the pleasantness of the trays on 100 mm VAS scales. The experimenters showed that all three measures of conditioning successively increased as conditioning was strengthened.

Furthermore, with every trial, participants were required to consume a food item. Unlike money which remains rewarding over time, food can lose its motivational salience with consumption (Davidson et al, 2018). Therefore, it would be counterproductive to repeat many trials using edible objects as a CS+. On the basis of previous experiments, four repetitions of our conditioning procedure was

considered optimal to maximise conditioning whilst minimising potential problems associated with satiation.

Consequently, a core aim of this experiment was to explore how motivational and attentional processing of novel cues are modulated by naturalistic appetitive conditioning across four consecutive trials, using subjective ratings and an EBA task respectively. In line with prior research by Papachristou et al. (2013 a gradual strengthening of subjective conditioned responses for a CS+ was expected across multiple trials. Based on the findings of Ghazizadeh et al. (2016), it was hypothesised that, whilst salience of the CS+ and CS- would be similar early on in the experiment, salience of reward-paired and neutral cues would diverge across multiple trials. Whilst attentional capture by a CS+ should *increase* across successive trials, conversely, salience for a novel stimulus paired with non-reward (CS-) should *decrease* as participants become more familiar with this stimulus.

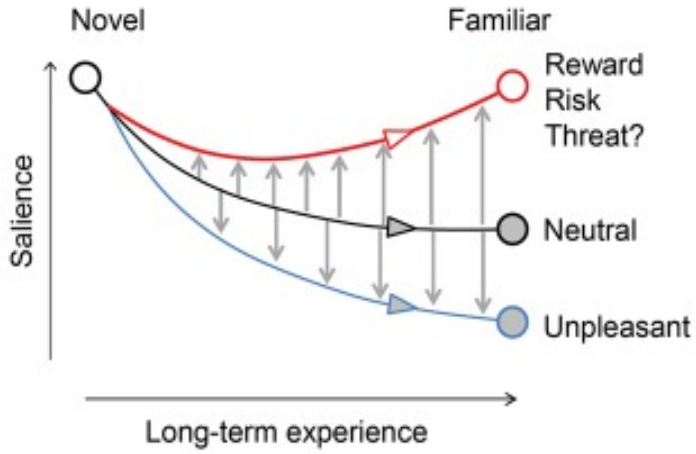


Figure 5.1 Representation of the effect of long-term experience to cues differing in valence and novelty on salience. Adapted from Ghazizadeh et al. (2016).

5.3 Method

5.3.1 Participants

Participants were recruited via online adverts and posters around the University of Liverpool campus and nearby areas. Eligible participants were required to be aged 18-40, have no food allergies or intolerances, like white chocolate and not be taking

medication known to affect attention or appetite. In order to avoid highlighting the importance of conditioning and drawing attention to the experimental aims, participants were informed that the study was investigating object perception and motivation. In total, forty participants completed the full experimental protocol. Ethical approval was granted by the University of Liverpool Ethics Committee. Participants were compensated for their time with course credit or £30 in high street vouchers.

5.3.2 Materials and measures

As in previous chapters, the materials and method are kept consistent wherever possible. Refer to General Methods [Chapter 2] for a full description of each measure. Methods unique to this particular chapter are described here in full.

5.3.2.1 Conditioned Stimuli

Two 3D novel geometric objects (yellow and orange) served as CSs. One object was made from white chocolate and became associated with the taste during acquisition when participants were asked to eat the object (CS+). The other was coated in plastic, signalling no reward associations (CS-). Colour-shape assignment was counter-balanced across participants. Each object weighed approximately 8g.

5.3.2.2 Self-report Measures of conditioning

Self-report measures of conditioning were collected immediately before each conditioning trial. Participants were presented with the CS object and asked to complete three explicit measures of conditioning before touching the object. Ratings were made using self-report 100 mm visual analogue scales (VAS), adapted from Papachristou et al. (2013). Questions were designed to capture conditioned cravings, CS-US expectancies, and changes in perceived liking (See Table 5.1 for question wording). The order of question type and stimulus presentation was randomised.

Table 5.1 Wording of questions and anchor points used on visual analogue scales presented during a self-report task where participants were required to rate the degree of pleasantness, cravings and expectancies elicited by a CS+ and CS-.

Measure	*Question*	*Anchors*
Cravings	*"When presented with this object, how strong is your craving for chocolate right now?"*	*"No craving at all" to "Extremely strong craving".*
US-Expectancy	*"When presented with this object, how strongly do you now expect to be invited to eat chocolate?"*	*"Certainly not" to "Certainly".*
Liking	*"How pleasant do you find this object?"*	*"Extremely unpleasant" to "Extremely pleasant"*

5.3.2.3 Emotional Blink of Attention Task

The EBA task design and procedure was largely consistent with Experiment 1-3 with minimal changes to the distractor categories and images (See General Methods [Chapter 2] for detailed description of the task). Distractor images were from one of four distractor categories in total: CS+, CS-, Dessert and Neutral. The experiment comprised 5 experimental sessions; a pre-conditioning session and four post-conditioning sessions completed immediately after each of the four conditioning trials (see Figure 5.2). Each session, comprised 4 blocks of 32 trials (128 trials in total). Participants took a short break between blocks. All images were randomly selected from their respective image banks. Distractors appeared equally across each session (8 presentations per block).

Visual stimuli

For consistency, the same filler images, rotated landscape targets and neutral distractors from Experiments 1-3 were re-used in the present study. The same 55 photographs of the CS+ and 55 photographs of the CS-, matched for background and luminance, used in Experiment 3 (Chapter 4) were selected again here. Finally, the 48 dessert images used in Experiment 2 (Chapter 3) (generously provided by Dr Graeme Davidson) were used again for the dessert distractor category. All images in the task were colour photographs, resized to 320 × 240 pixels.

5.3.2.4 Questionnaires and additional measures

The Dutch Eating Behaviour Questionnaire (DEBQ; Van Strien, Frijters, Bergers & DeFares, 1986). Food Neophobia Scale (FNS; Pliner & Hobden, 1992), BIS/BAS Scale (BAS-RR) (Carver & White, 1994) and the 8-item Brief Sensation Seeking Scale (BSSS-8; Hoyle et al, 2002) were used to provide descriptive information about the sample (see Chapter 2 for a full description).

5.3.3 Procedure

Participants were instructed to abstain from eating or drinking any calorific food or beverage for at least two hours prior to the experiment. Testing took place from mid-morning (between 11:00 - 14:00) or mid-afternoon (approx. 14:00 - 17:00). Upon arrival at the laboratory, participants were seated in an individual booth, gave full informed consent, and then completed the screening procedure (see Chapter 2).

In total, participants completed four conditioning trials. The first trial began by asking participants to complete appetite ratings, and a pre-conditioning EBA session (as described in Chapter 2). Participants were then presented with either the CS+ or the CS-, in a counterbalanced order. The CS+ was presented on the table in front of participants, on a small white plate. The CS- was presented in a small white plastic box for consistency. Participants were then asked to provide self-report ratings of cravings, expectancies and liking elicited by the appearance of the object alone object, using the three VAS scales described above. Participants underwent the acquisition phase, being asked to handle, smell and touch the first CS for two minutes (and eat the CS+) then rate the sensory characteristics of the object (including taste for the CS+ only) on 100 mm VAS scales. After a 90 second break, they were then presented with the other CS and repeated the three self-report scales and a two-minute acquisition phase. The EBA task was repeated after each of the four conditioning trials, and appetite and self-report conditioning measures were collected prior to the final three acquisition phases. Participants were given a ten-minute break after each run of the EBA, when they were instructed to remain in the lab but were free to relax, use their phones or read magazines provided. After all four trials were completed, participants completed a computerised VAS task to measure perceived valence of the images used in the EBA (See Chapter 2 for full description of VAS task), a battery of questionnaires (DEBQ, FNS, Brief-SSS and BIS/BAS and

had their height and weight measured using scales and a stadiometer. Awareness of the CS-US contingencies was assessed by asking participants to recall the colour-shape combination associated with the CS+ and CS-. Participants were asked to write a brief summary of what they thought were the aims of the experiment to assess awareness. The full experimental procedure lasted approximately three hours. For a schematic representation of sequencing of the four conditioning trials, see Figure 5.2.

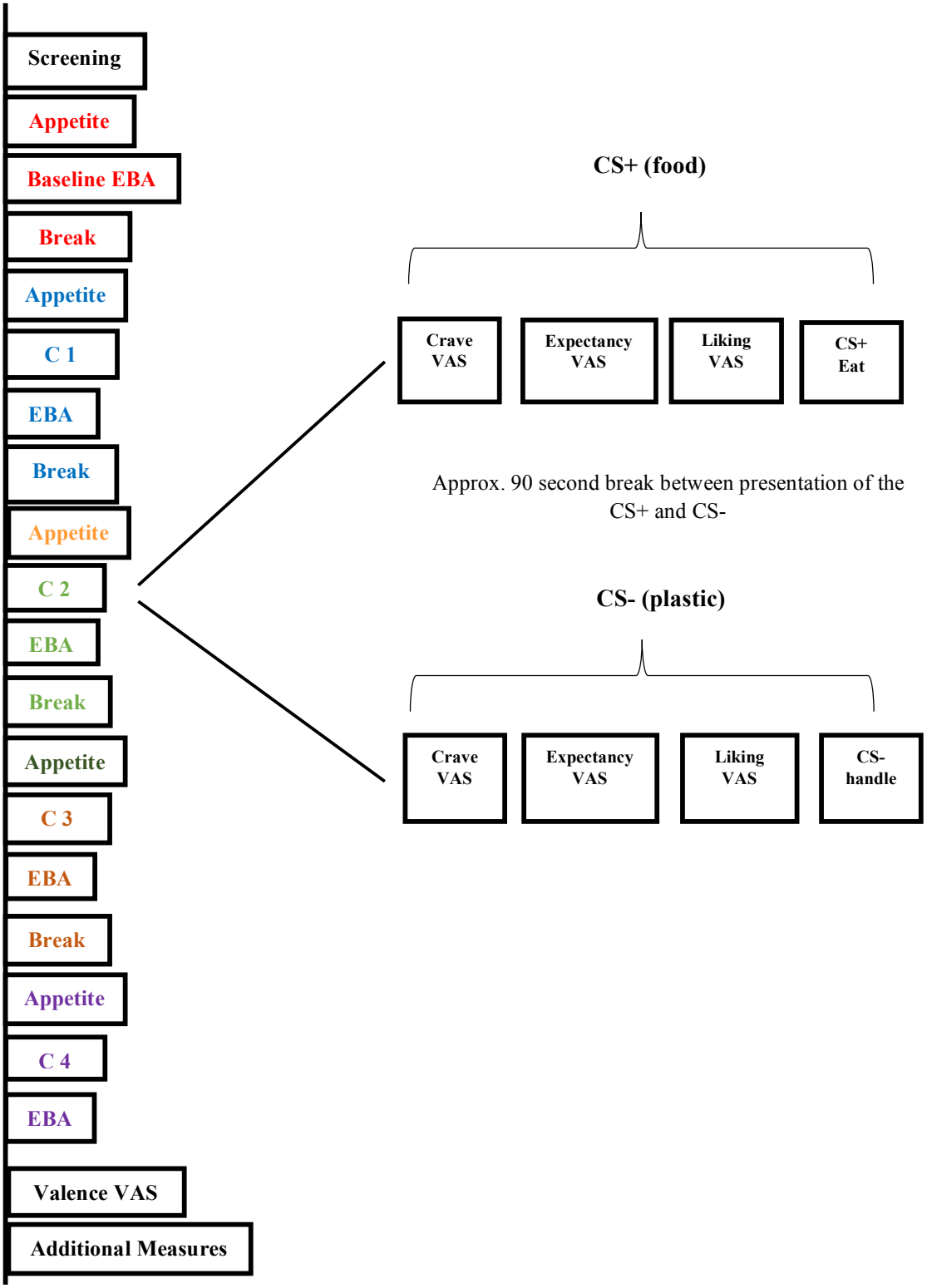


Figure 5.2 Overview of main experimental procedure depicting four conditioning trials and associated self-report and attentional measures. C 1 - 4 refer to the conditioning trials, including the presentation of the CS stimuli, self-report conditioning VAS (cravings, expectancies and liking) and the acquisition phase. Order of presentation of CSs and self-report questions was counterbalanced across participants and trials. Different text colours are used to depict different phases of the experiment: red = baseline; blue = trial 1, yellow = trial 2, brown = trial 3, purple = trial 4, black = additional steps.

5.4 Data Preparation

Of the 40 participants who completed the full experiment, 3 were removed for particularly poor performance on the EBA task. These participants consistently performed below chance level across the experiment. These participants were excluded from all analyses leaving a final sample of 37 participants.

Although a Shapiro-Wilks test highlighted some deviations from normality, after assessing Q-Q plots and histograms these were deemed to be minor – in line with Ghasemi and Zahediasl (2012). These authors also argue that departures from normality are unlikely to be problematic for sample sizes greater than 30, as parametric tests are relatively robust. Consequently, parametric tests were conducted as per our original analysis plan. Where the assumption of sphericity was not met, Greenhouse-Geisser corrections were applied. *Post hoc* tests are reported with Bonferroni corrections for multiple comparisons.

5.5 Results

5.5.1 Participant characteristics

Table 5.2 provides a summary of participant characteristics in the present sample. Participants were typically early – mid-twenties, reflective of the predominantly student sample. On average, participants had a normal body mass index (BMI). In terms of personality variables, several outliers were identified but their exclusion did not alter the pattern of results so they remain in the full analysis.

Levels of food neophobia were similar to that reported in a comparable sample of European university students (mean ± SD: 29.39 ± 10.07; Fenko et al., 2015) Scores ranged from 11 - 54 and, using the cut-offs proposed by Previato and Behrens (2015), 62% of the sample can be classified as neutral (16.5-38.5), 22% as Neophillic (<16.4) and 16% as neophobic (>38.6).

Eating attitudes were also consistent with mean scores of a sample of Dutch undergraduates on DEBQ subscales restraint, emotional eating and external eating (mean ± SD were: 2.47 ± 0.88; 2.48 ± 0.71 and 3.13 ± 0.51, respectively; van Strien, Herman & Anschutz, 2011).

BAS-RR scores were comparable to those of moderate scorers in a non-clinical sample (16.83 ± 2.26; Alloy, et al. 2006). The Brief-SSS: Total was also

similar to that of a UK University-based convenience sample (3.01 ± 0.59; Eachus, 2004).

All participants were aware of the CS-US contingencies. No participants accurately identified the full aims of the experiment. However, four participants mentioned the effect the presence of food-paired images in the image streams had on attention, whilst three mentioned the effect of conditioning on pleasantness or cravings for a food-paired object. Removal of these individuals did not affect the pattern of results.

Table 5.2 Participant demographics. (Means ± SD)

Gender	
Female N (%)	20 (54%)
Age	24.41 ± 5.18
BMI	24.18 ± 3.75
DEBQ	
Restraint	2.58 ± 0.69
Emotional	2.70 ± 0.87
External	3.53 ± 0.67
FNS	25.30 ± 11.43
BIS/BAS	
BAS-RR	16.08 ± 2.82
Brief-SSS	3.34 ± 0.76

BMI = body mass index, DEBQ = Dutch Eating Behaviour Questionnaire; FNS = Food Neophobia Scale; BIS/BAS = Behavioral Inhibition System (BIS) and the Behavioral Activation System (BAS); Brief-SSS = Brief Sensation Seeking Scale. As the DEBQ has a distinct three-factor structure a total score was not computed.

5.5.2 Self-report measures

5.5.2.1 Appetite

A one-way repeated measures ANOVA revealed that appetite varied significantly over time, $F(2.17, 78.16) = 4.21$, $p = .016$, $\eta_p^2 = .11$ (See Figure 5.3). The change in appetite scores across the experiment was best described by a quadratic function, $F(1, 36) = 7.52$, $p = .009$, $\eta_p^2 = .17$. Repeated contrasts showed that appetite increased sharply from T1 to T2, $F(1, 36) = 14.29$, $p = .001$, $\eta_p^2 = .28$. T1 reflects baseline measures at the beginning of the experiment and T2 reflects the moment immediately before the first conditioning trial. Therefore, participants had a period of approximately 30 minutes without any food in which hunger steadily rose. Subsequently, there was no further change in appetite scores, which remained high across the rest of the experiment, ($ps \geq .503$). The quantity of chocolate consumed across the study (32.67 g ± 5.07) did not therefore appear to satiate participants.

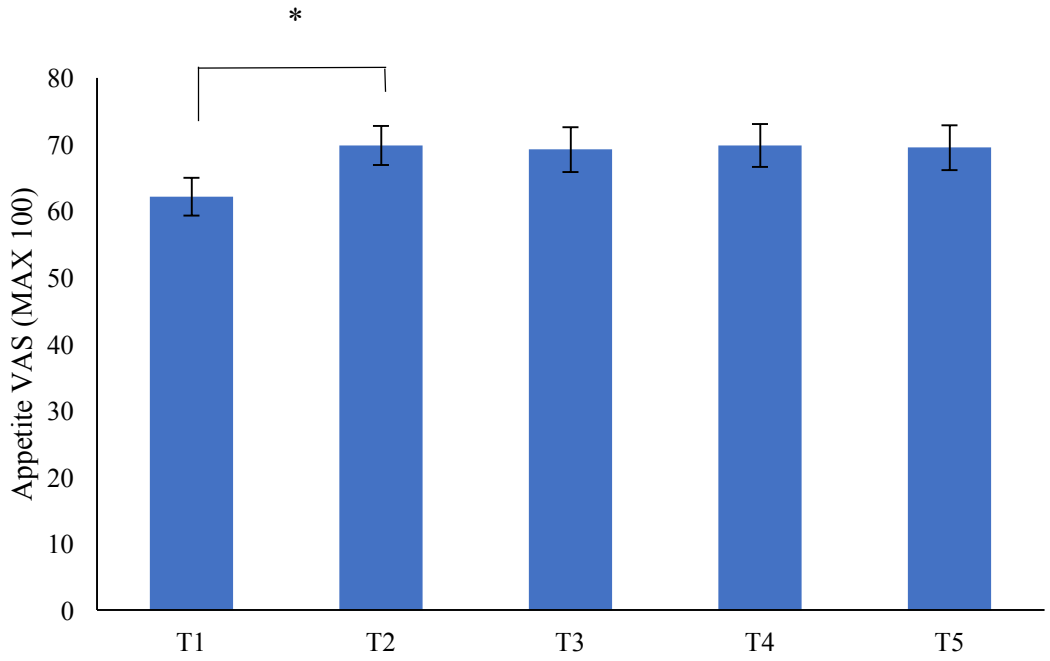


Figure 5.3 Average appetite ratings at the start of the experiment (T1) and immediately before each of four conditioning trials (T2 – T5). *$p<.05$

5.5.2.2 Conditioning

Taste ratings and Consumption

Taste ratings did not differ between the four conditioning trials, $F(1.75, 61.22) = .79$, $p = .442$, $\eta_p^2 = .02$, and were consistently high across the experiment (see Table 5.3 for descriptives). Two outliers were identified that liked the taste of the chocolate less than average (2.5 SDs outside the mean). Removal of these participants did not change the pattern of results so their data were kept in the final analysis. All remaining participants gave a total average score ≥ 57. On average, participants consumed 32.67 ± 5.07 g across the experiment, with 82% of participants choosing to consume all the chocolate provided. Those that chose to leave some of the chocolate ate a minimum of 21.20 g across the experiment.

Table 5.3 Average taste ratings for the CS+ (white chocolate) across four conditioning trials. (Means ± SD). C = Conditioning trial.

	Mean (±*SD*)	Min	Max
C1	77.11 (*15.27*)	35	98
C2	80.03 (15.56)	28	99
C3	79.68 (16.53)	23	100
C4	77.08 (21.43)	10	100

Conditioned Expectancies

A 4 (time) × 2 (CS type) repeated measures ANOVA revealed a significant interaction between stimulus type and time point on chocolate expectancy ratings, $F(3, 108) = 37.26$, $p = .001$, $\eta_p^2 = .51$. As Figure 5.4 illustrates, there was clear evidence of differential appetitive conditioning for expectancies. Prior to conditioning, expectancy ratings were similar for the CS+ and CS-, but after

conditioning the CS+ elicited significantly greater expectancies for chocolate than the CS- at T2, T3 and T4 (*p*s < .001).

For the CS+, there was a significant linear trend, $F(1, 36) = 113.83$, $p = .001$, $\eta_p^2 = .76$, with expectancies for chocolate increasing across the experiment. Repeated contrasts revealed a sharp increase from T1 to T2, $F(1, 36) = 63.51$, $p = .001$, $\eta_p^2 = .64$. The difference between remaining successive trials (T2 vs T3 and T3 vs T4) failed to reach significance (*p*s > .126)

For the CS- there was a significant linear trend in the opposite direction, with chocolate expectancy gradually declining over the course of the experiment; $F(1, 36) = 13.79$, $p = .001$, $\eta_p^2 = .28$.

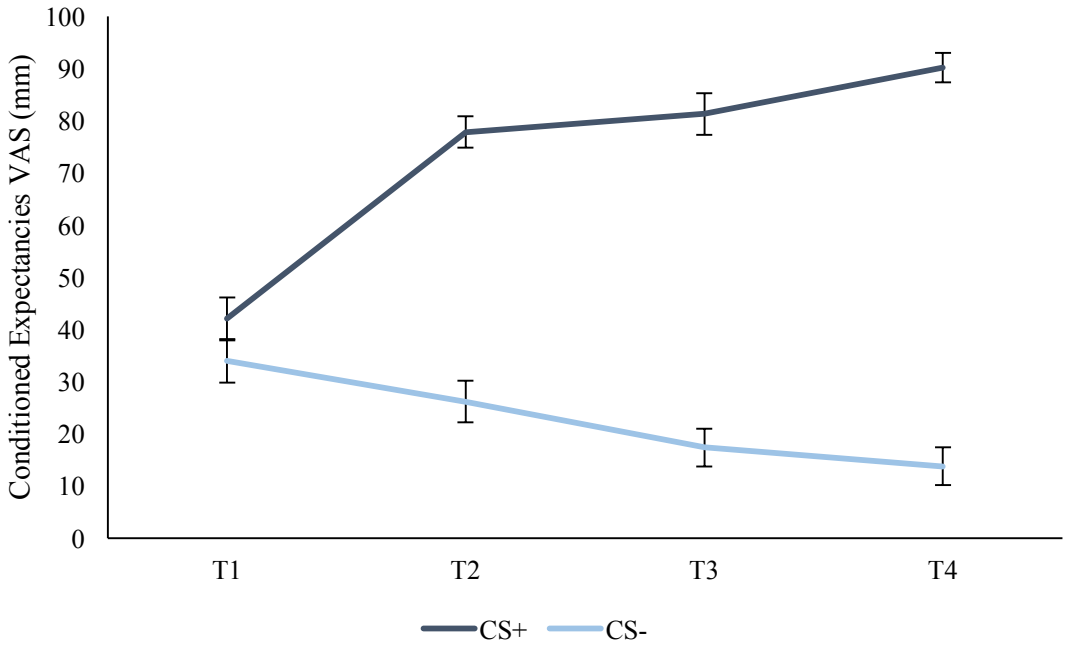


Figure 5.4 Mean expectancies (for chocolate) scores in response to the CS+ and CS− measured prior to each of four conditioning trials. Bars represent standard error.

Conditioned Cravings

There was a significant interaction between stimulus type and time point on conditioned cravings, $F(3, 108) = 6.80$, $p = .001$, $\eta_p^2 = .16$. After correcting for multiple comparisons, the difference between cravings elicited by the CS+ and CS- at baseline failed to reach significance ($p = .029$). However, the CS+ elicited

significantly greater cravings than the CS- at each time-point post-conditioning (T2, T3 and T4) (*p*s < .001).

Polynomial contrasts revealed that the simple effect of time on conditioned cravings elicited by the CS+ was best described by a quadratic function; $F(1, 36) = 17.43$, $p = .001$, $\eta_p^2 = .33$. Cravings increased sharply between T1 and T2, $F(1, 36) = 18.60$, $p = .001$, $\eta_p^2 = .34$, and then levelled off. Further changes (T2 vs T3 and T3 vs T4) failed to reach significance (*p*s ≥ .112). Cravings in response to the CS- did not change across the experiment.

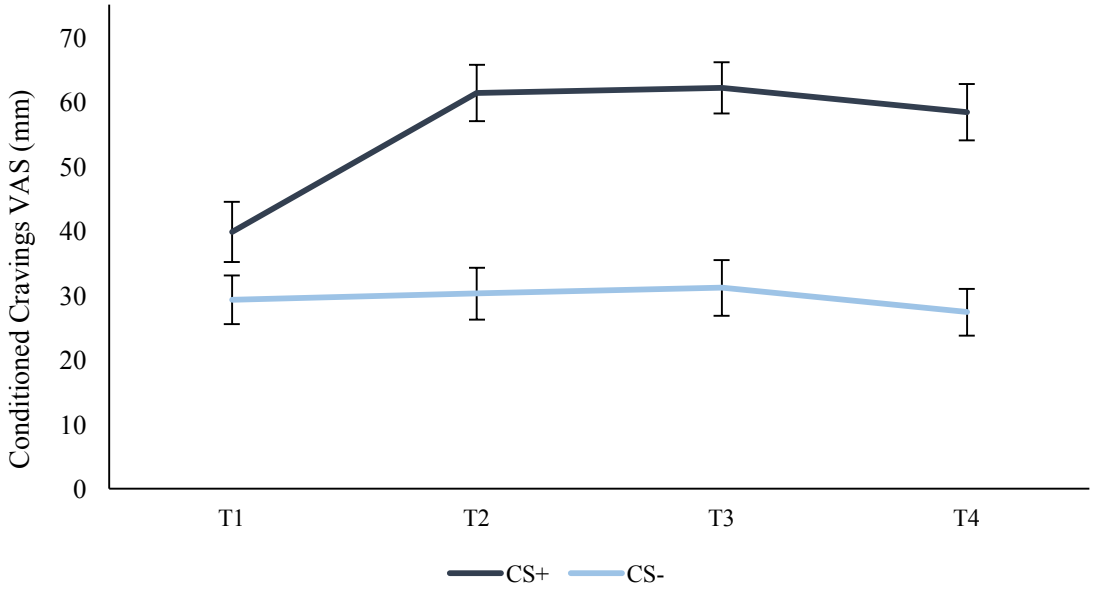


Figure 5.5 Mean craving (for chocolate) scores in response to the CS+ and CS− measured prior to each of four conditioning trials. Bars represent standard error.

Conditioned liking

A 2 (CS Type) × 4 (Time) repeated measures ANOVA revealed a significant interaction between stimulus type and time point on liking, $F(2.39, 86.01) = 18.32$, $p = .001$, $\eta_p^2 = .34$ (see figure 5. 6). Paired t-tests revealed that the CS+ was liked significantly more than the CS- at T2, T3 and T4 (*p*s < .001), whilst at T1 (pre-conditioning) they were liked equally ($p < .384$).

With regards to the CS+ there was an upward linear trend, $F(1, 36) = 31.90$, $p = .001$, $\eta_p^2 = .47$, with a quadratic component, $F(1, 36) = 11.54$, $p = .002$, $\eta_p^2 = .24$. There was a significant increase in liking from T1 (prior to conditioning) to T2, $F(1,$

36) = 17.51, p = .001, η_p^2= .33. The change across later trials (T2 vs T3 and T3 vs T4) failed to reach significance (ps ≥ .06).

Regarding the CS-, there was a significant downward linear trend in liking across the experiment, $F(1, 36) = 11.14$, p = .002, η_p^2= .24. A significant decrease in liking occurred between T1 and T2, $F(1, 36) = 4.13$, p = .049, η_p^2= .10 and T3 and T4, $F(1, 36) = 10.63$, p = .002, η_p^2= .23.

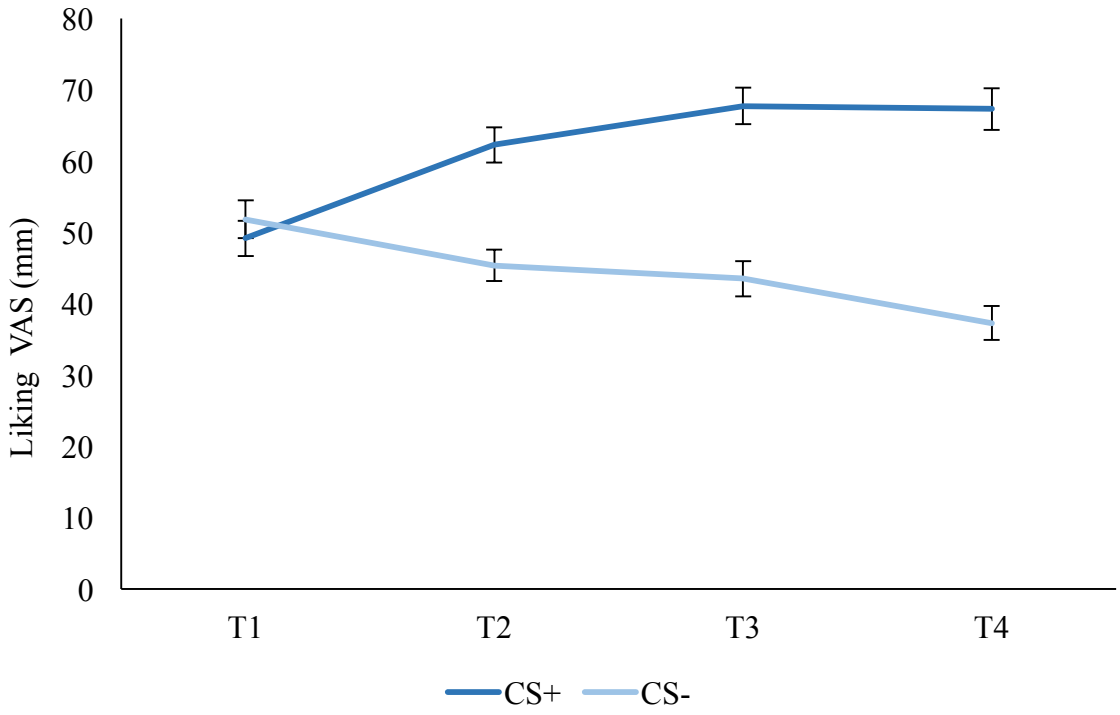


Figure 5.6 Mean liking scores for the CS+ and CS- measured prior to each of four conditioning trials. Bars represent standard error.

5.5.3 Attention: EBA Task

5.5.3.1 Response Accuracy (%)

Accuracy scores, representing the percentage of correct trials for each distractor type, are summarised in Figure 5.7: a lower score represents greater attention capture by the distractor images. A 4 (distractor type) × 5 (time point) repeated measures ANOVA revealed a significant main effect of distractor type, $F(3, 108) = 18.58$, p = .001, η_p^2 = .34. The main effect of time and the interaction term were non-

significant. Overall the dessert, CS+ and CS- images were significantly more distracting than neutral images, irrespective of time point (*p*s < .001).

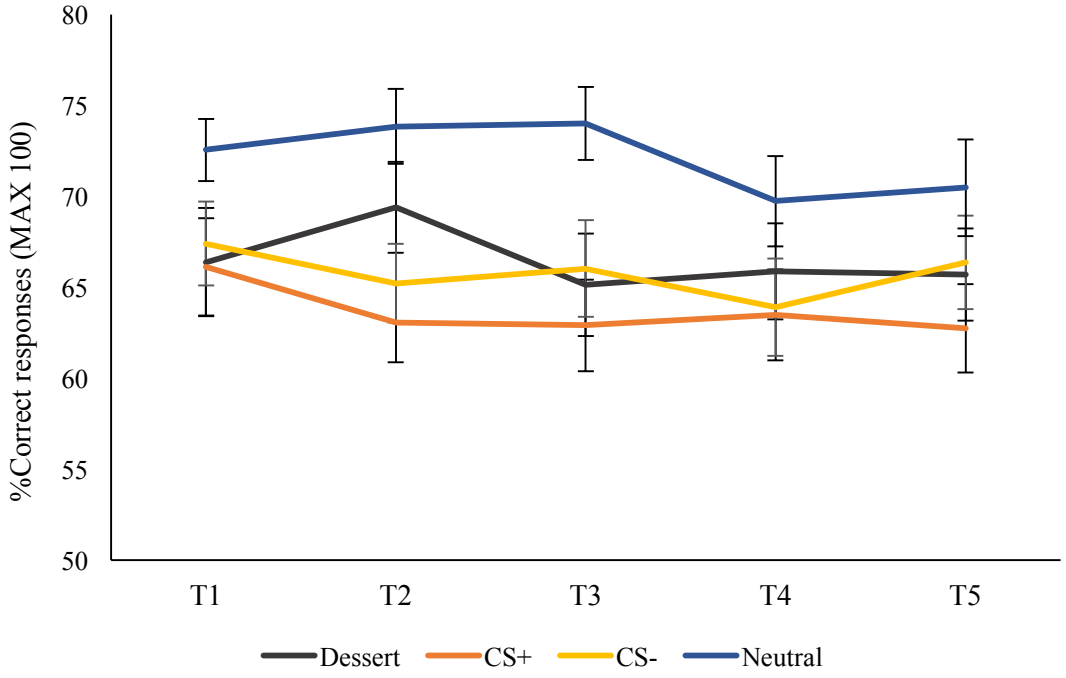


Figure 5.7 Mean target detection accuracy on an EBA task before conditioning (T1) and after each of four conditioning trials (T2-T5). Bars represent standard error.

5.5.3.2 Exploratory analysis

In an attempt to understand these equivocal findings, further exploratory analysis was conducted. Additional repeated measures ANOVA were conducted and significant effects were followed-up with uncorrected *post hoc* pairwise comparisons as, in this case, Bonferroni corrections would likely be overly conservative.

Fatigue and practice effects may have been prominent in the present study due to the lengthy and repetitive testing schedule. Evidence suggests that habituation to salient stimuli can occur across multiple trials, reducing the magnitude of attentional bias effects (Staugaard, 2009). Furthermore, Evidence from the threat-related attentional bias literature suggests that through repeated practice, participants may develop more effective means of completing attention tasks and become better at exerting top-down control (Mogg, Waters & Bradley, 2017).

As a result, it was predicted that performance on the EBA task may have been most strongly impaired by distractors in early trials and thus, analysis was focused on the first half of trials per session only when, theoretically, habituation and top-down control over responses should be less prominent. However, this did not change the pattern of results.

In addition, although the interaction term was non-significant, a series of one-way repeated measures ANOVAs were conducted to explore the simple effect of time point for each distractor type, in case statistical power to detect significant effects was lacking. There was a trend towards significance for the effect of time on performance for trials where the neutral distractor was present, $F(4, 144) = 2.42$, $p = .051$, $\eta_p^2 = .06$. This was driven by a drop in accuracy from T3 (74.01 ± 12.37) to T4 (69.74 ±15.26), ($p = .034$), perhaps reflective of fatigue or boredom beginning to hinder performance in later sessions.

Based on this data it would appear that by session four, participants were fatigued, perhaps contributing to a drop in accuracy . In order to minimise the impact of fatigue effects on the data, session four and five were removed from the analysis and a further 3 (time) × 4 (distractor type) repeated measures ANOVA was conducted, focusing solely on T1-T3. Again this revealed a significant main effect of type, $F(3, 108) = 15.65$, $p = .001$, $\eta_p^2 = .30$, but neither the main effect of time or the interaction were significant. This was also true when analysis was focused on T1 vs T2 or T1 vs T5, suggesting that effects of repeated practice, or task fatigue are unlikely to account for the lack of significant effects.

5.5.3.3 Reaction times

A further 5 × 4 repeated measures ANOVA revealed a significant main effect of time, $F(2.14, 76.89) = 11.24$, $p = .001$, $\eta_p^2 = .24$, but no main effect of distractor type, or a time × type interaction ($ps > .05$). Reaction times decreased across the experiment in a linear fashion, $F(1, 36) = 18.76$, $p = .001$, $\eta_p^2 = .34$ from T1 (Pre-conditioning) (274.37 ± 102.20) to T5 (Post-conditioning Trial 4) (229.95 ± 93.52).

5.5.3.4 Image valence (VAS)

There was a main effect of image type on valence ratings for the EBA images, $F(3.05, 106.61) = 20.27$, $p = .001$, $\eta_p^2 = .37$ (see figure 5.8). Overall, the CS- images were rated as significantly less pleasant than the other image types, $ps < .001$. The

dessert and filler images were also rated as significantly more pleasant than the neutral and CS+ images, *p*s < .009.

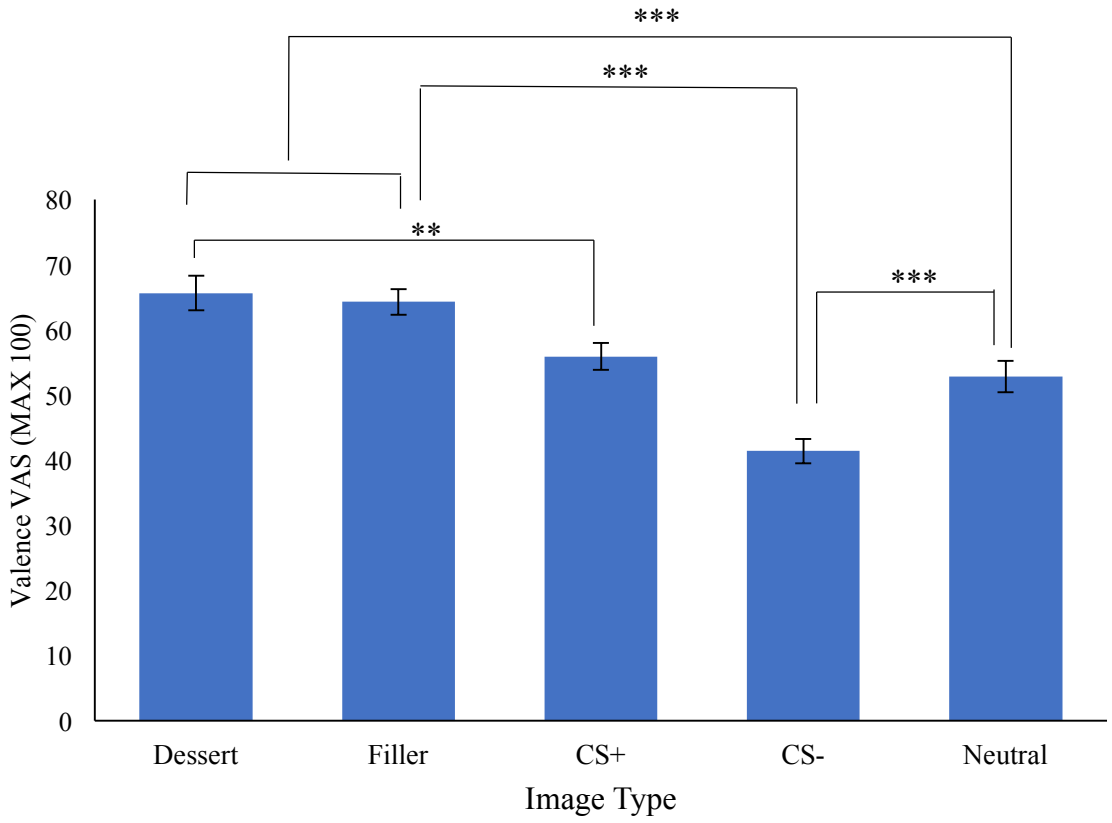


Figure 5.8. Mean valence ratings of a random sample of images from the EBA task, measured on 100 mm VAS, both before and after conditioning. Bars represent standard error. *p<.05, **p<.01, ***p<.001.

5.5.4 Associations with individual differences

Correlations were conducted between individual differences (e.g., eating attitudes, incentive sensitivity) and key outcome measures; difference scores (T4 – T1) for self-report measures (craving, expectancy and liking) and difference scores (T5 – T1) for attentional capture on the EBA task. After applying corrections, no significant correlations were identified (see Appendix E, Table E1 for correlation matrix).

5.6 Discussion

In light of the equivocal findings outlined in Chapter 3, the purpose of the present study was to measure the acquisition of subjective conditioned responses (craving, expectancy and liking) across four appetitive conditioning trials and to measure automatic capture of attention as an objective, implicit marker of conditioning. The results support the rapid, differential acquisition of subjective CRs, yet there was no evidence that this reward learning modified attentional capture.

After conditioning with our novel CS-US compounds, participants liked the CS+ significantly more than the CS-, and showed enhanced cravings and expectancies for chocolate in response to the CS+. These changes suggest successful differential appetitive conditioning, in line with previous findings from Papachristou et al. (2013) and highlighting the validity of our conditioning procedure.

Furthermore, these data suggest that, in fact, the greatest change in ratings occurred after the first conditioning trial. All three measures saw an abrupt increase in ratings for the CS+ after just one trial. Whilst expectancies continued to increase past trial one at a more gradual pace, both liking and cravings for chocolate levelled off, suggesting that additional trials do little to strengthen the CS-US relationship. This adds further strength to findings presented earlier in this thesis, suggesting that differential appetitive conditioning can occur rapidly under certain circumstances.

This pattern of results appears to challenge the assumption of traditional learning paradigms such as the Rescorla-Wagner (1972) model that learning is a gradual process where the associative value of a CS changes incrementally across repeated conditioning trials. Instead, these data indicate a more abrupt style of learning as proposed by Gallistel, Fairhurst & Balsam (2004), whose data suggest that learning typically occurs in a more 'all or none' fashion. In their research, subjects typically demonstrated abrupt learning in as little as a single trial, often with a rapid, step-like increase in learning curves from an untrained level of responding to a level seen in the well-trained subject, with no further increase in learning evident with additional trials. The authors suggest that once a certain learning threshold has been reached, responding is at a maximum and cannot be further strengthened. Some failures to replicate their work had called into question the possibility that all Pavlovian conditioning will result in this pattern of learning (e.g., Harris, 2011), but it appears that our naturalistic conditioning paradigm is sufficiently potent to promote rapid single-trial learning. Domjan, Cusato & Krause (2004) argue that the

use of more ecologically valid CSs can uncover novel learning mechanisms. In this way, the paradigm described here may reflect a special case – specific to eating behaviour, which perhaps evolved in our ancestral past when the discovery of new foods was of great significance for survival. Further research with different experimental paradigms will be needed to explore this possibility further.

Based on the apparent strength of this specific procedure on subjective measures, it is perhaps surprising that we did not observe the expected pattern of results in our attentional data. As expected, in comparison to neutral distractors, dessert images were consistently more salient across the experiment, most likely due to the high degree of motivational salience associated with such palatable foods (Davidson et al., 2018). However, both novel, previously unseen CS+ and CS- distractors captured attention to the same extent as desserts overall, even *before* conditioning took place.

While the pre-conditioning capacity of the novel stimuli to capture attention was not predicted – and does not match our previous findings, a close reading of the literature suggests a possible explanation – that novel stimuli capture attention independently of reward (e.g., Yang, Chen & Zelinsky, 2009). The unusual nature of the conditioning procedure, along with the novelty of the objects may have enhanced the salience of the CS stimuli. Dopamine neurons are activated by novelty, even in the absence of reward, affecting both attention and memory (Horvitz, 2000). Additionally, novelty can enhance low-level visual perception, which is triggered by the overall significance of the stimuli (Schomaker & Meeter, 2013). It is argued that, in line with the boost and bounce theory of temporal attention (Olivers & Meeter, 2008), that novelty is so significant that it can trigger a transient attentional *boost* of novel distractors and induce an EBA, in much the same way as emotionally or motivationally significant distractors.

Furthermore, novelty confers an exploration bonus, which ensures continued exploration of a novel stimulus or environment (Dayan & Sejnowski, 1996), via enhanced dopaminergic activity in the midbrain (Wittmann, Bunzeck, Dolan & Düzel, 2007). Therefore, the high and persistent salience of the novel objects seen here may reflect this mechanism: novel items may remain salient, at least in the short term, to promote further exploration and learning about the possible associated rewards or punishments. The fact that both novel objects, even the CS-, remained

equally as salient as the dessert images highlights the strength of this mechanism to potentially override any effects of reward learning on attention.

It must be re-iterated that this is the first time with this experimental paradigm that the CS+ and CS- objects have captured attention more strongly than neutral stimuli prior to the first conditioning trial. Chapter 3 presented a series of studies which suggested that the CS+ objects became more salient than neutral distractors only after conditioning took place. The type of images and experimental protocol were consistent with previous experiments, so the reasons for the disparities in preconditioning responses between experiments are unclear.

Based on the work of Ghazizadeh et al. (2016) some divergence between the CS+ and CS- over post-conditioning trials was expected. Their work in primates showed that reward paired cues increased in salience, whereas mere exposure to novel objects resulted in a reduction in salience. The present data failed to reveal such a pattern; despite repeated exposure to the novel CS- objects, salience remained consistently high, suggesting that novelty-based salience did not decrease as participants became more familiar with the objects. Moreover, according to the Mackintosh model (1975), capture by reward-paired cues will increase with experience, as reward expectancy increases. However– yet, again, no such change was evident in the present data.

The fact that different patterns of responding were observed on subjective ratings compared to the attention task is consistent with the view that value and salience are dissociable constructs (Kahnt, Park, Haynes & Tobler, 2014). Whilst value refers to the meaning associated with a stimulus (e.g., reward or punishment) and the associated valence (positive or negative), salience refers to the ability of a stimulus to capture attention, and simply refers to the overall significance of the stimulus, regardless of its predicted outcome (Kahnt & Tobler, 2017).

Although both value and salience are related, they should arguably be treated as separate constructs. For example, it cannot be assumed that a stimulus is well-liked just because it is salient. Salience is difficult to define and can be attributed to many different stimulus features, including perceptual salience (e.g., brightness, contrast, colour; for a review see Wolfe & Horowitz, 2004), or associability with rewarding outcomes (e.g., Anderson et al., 2011a). Thus, there may be many sources of salience, and objects encountered in the environment may capture attention even if experience renders them disliked or they lack associations with reward. This

complexity highlights the importance of considering multiple measures when attempting to explore the influence of reward learning on attention, perception and behaviour.

A number of limitations associated with the present study must be acknowledged. Although the small number of trials selected for this experiment was theoretically justified (Papachristou et al., 2013), it is possible that the time frame was insufficient to create any observable change in attentional processing. Ghazizadeh et al. (2016) studied primates over five days and exposed them to over fifty viewings per object. Although their study design was significantly more complex, this does suggest that the changes they observed occurred over a longer period than our design allowed. Over just four trials, it is plausible that novelty and uncertainty remained high. Hence, although reward learning rapidly altered explicit markers of conditioning, this learning could not be teased apart on the emotional blink of attention task. Effects of novelty alone may thus persist despite changes in the perceived value of the objects.

Additionally, despite using only four trials, a major limitation of this study was the repetitive and lengthy protocol participants were required to complete. Testing spanned across a three-hour session and was very repetitive in nature. Although regular breaks were provided, it is possible that participants still felt bored and fatigued by the procedure and, therefore, their responses towards the end of the experiment may have been affected. Furthermore, the time costs involved with this experiment meant that additional trials would have been impractical. A different experimental design, perhaps with shorter test sessions across multiple test days could provide greater insight in to the underlying processes. Future research should carefully consider these factors, and more fully explore the time course of learning and experience on attention.

In conclusion, the present study again demonstrates that appetitive conditioned responses with chocolate as a CS+ can be learned rapidly, with only one learning trial. Subjective measures of cravings, US-expectancies and liking revealed clear evidence of differential appetitive conditioning using a naturalistic conditioning procedure. Across four learning trials, the largest change in self-report ratings occurred after the first, with little evidence that further trials strengthened these responses. This pattern suggests that, under some circumstances, appetitive conditioning can occur abruptly and further strengthens the validity and utility of this

procedure for investigating appetitive conditioning processes in humans. In contrast, attentional processing of novel objects appears to be unaffected by learning or experience, at least in the short term. Novel items which are potentially relevant for survival may automatically capture attention regardless of learned experiences, perhaps in anticipation of potential reward or harm. This experiment questions the role of learning and experience in attentional processing of reward related cues and highlights the importance of considering both value and salience in this process.

Chapter 6

Stimulus Generalisation in Single-trial Conditioning and the Role of Stimulus Novelty.

6.1 Abstract

The ability to generalise from past experiences is an essential survival skill. Through a Pavlovian conditioning process – stimulus generalisation, learning can spread to stimuli with some shared resemblance to a conditioned stimulus (CS). As the majority of research has focused on conditioning in relation to fear and anxiety, tasks typically use aversive stimuli such as pain as the unconditioned stimulus (US). Here we present a paradigm adapted from the fear conditioning literature to examine whether stimulus generalisation can occur with appetitive stimuli, specifically using palatable food (white chocolate) as an unconditioned stimulus (US). An edible object was paired with chocolate (CS+), which participants were asked to eat, and an inedible object was paired with plastic (CS-) which participants simply handled. Generalisation included six intermediate objects which varied in their similarity to the CS+ and CS−. In order to explore the role of stimulus novelty, participants were assigned to either a familiar condition (n=26) or a novel condition (n =23) where the CS+ and CS– respectively had either a familiar or novel appearance. Again, self-reported ratings (cravings, US-expectancies and liking) and attentional capture (EBA task) were the primary measures of interest. Self-report data indicated that acquisition of conditioned responses for the CS+ generalise to stimuli based on their perceptual similarity to the CS+, with those most similar showing the greatest generalisation. EBA data indicate that, at an attentional level, any newly experienced cue will acquire salience, regardless of reward associations, perhaps owing to the unusual nature of this naturalistic single-trial conditioning procedure. Our findings demonstrate how food-cue reactivity (i.e., cravings) to new foods may develop based on a shared similarity to other palatable foods with which the individual has previously had a rewarding experience. The present study highlights the utility of this procedure for exploring the role of generalisation in the emergence of appetitive conditioning and its role in human eating behaviour.

6.2 Introduction

Learning to overeat

Learning-based models of eating behaviour posit that food cue reactivity is a conditioned response (CR) (Boswell & Kober, 2015). Via Pavlovian conditioning, external or interoceptive cues (conditioned stimuli; CS) become associated with ingestion of food (unconditioned stimulus; US) and subsequently elicit a CR, such as cravings that may lead to overeating and subsequent weight gain. Typical conditioning paradigms only test CRs in response to the exact stimuli that were present during learning. However, in today's obesogenic environment, food cues are highly abundant, widely accessible and aggressively promoted (Blundell et al., 2005). In the visual domain alone, the appearance of food is constantly evolving, and we are exposed to a vast number of food logos and packaging styles each day, with more than 80% of all US grocery products being branded (Story & French, 2004). The sight or smell of a new food product is frequently evident even in the absence of its ingestion. Hence, the number of cues with the potential to become associated with food is almost unlimited. An important question then is whether CRs are only elicited by a specific CS which has been directly paired with an appetitive US (e.g., sweet taste), or if stimuli that are similar to the CS in some way could also elicit CRs.

Stimulus Generalisation

From an evolutionary perspective, the ability to generalise seems beneficial for promoting efficient learning. The spread of reinforcement from one conditioned stimulus to another similar stimulus is a well-documented phenomenon known as stimulus generalisation (Catania, 1998). Stimuli which share some similarity with the CS are able to elicit a conditioned response similar to that elicited by the original CS, despite never being presented directly alongside the US (Cuvo, 2003).

For example, in a classic experiment by Watson & Rayner (1917), an infant known as Little Albert underwent fear conditioning; a white rat was consistently paired with an aversive loud noise. They observed that Albert exhibited a conditioned fear response in response to white rats in the absence of the aversive noise. However, he also showed a similar fear response to a variety of white fluffy stimuli, such as the experimenter's hair, cotton balls and a rabbit. It seems that not

only had little Albert learned to associate white rats with an aversive noise, he had also generalised this fear to stimuli which were white and fluffy.

That experiment was hugely influential within the study of fear and anxiety, and this is arguably the area where stimulus generalisation is most widely studied. Lissek et al. (2008) were the first to present a novel fear-potentiated startle paradigm whereby conditioning and generalization stimuli were images of ten hollow rings, increasing incrementally in size (from 2 to 5.6 inches), creating a similarity continuum from the CS+ to CS-. The researchers were able to demonstrate a generalisation gradient, where the generalisation stimuli most similar to the CS+ elicited the greatest fear, and a continuous decrease in generalisation as stimuli became less similar. More recently this paradigm has been replicated and extended using photographs of human faces as conditioned threat (CS+) or safety (CS-) cues, with intermediate generalisation photographs varying in similarity systematically from the CS+ to CS- (Haddad, Xu, Raeder & Lau, 2013). Again, acquired fear of the CS+ generalised to intermediate stimuli in proportion to their similarity to the CS+.

To date, there has been no research which has directly assessed this generalisation phenomenon with food as an appetitive reinforcer, thus its implications for human eating behaviour are unclear. Consequently, a core aim of this experiment was to adapt the novel paradigm described by Haddad et al. (2013) for use with appetitive stimuli to assess whether stimulus generalization occurs for explicit measures of conditioning – in this case CS pleasantness, cravings and expectancies.

It was hypothesised that, in line with findings from the fear conditioning literature, a generalisation gradient will emerge with conditioned cravings, expectancies and pleasantness being greatest for the CS+ and the most similar generalisation stimuli, and decreasing as they become increasingly similar to the CS-.

Attention as a measure of stimulus generalisation

A broad body of research has consistently demonstrated that reward learning can enhance the salience of an otherwise neutral cue via Pavlovian conditioning processes (Bucker & Theeuwes, 2017). After pairing with reward, previously neutral stimuli subsequently capture attention (Della Libera & Chelazzi, 2009), even when they are irrelevant to the current task (Anderson, Laurent & Yantis, 2011a).

Therefore, attentional capture can serve as an implicit measure of the degree of incentive salience acquired by conditioned stimuli.

Chapter 4 presented a novel experiment in which attentional capture was enhanced for both an appetitive CS (novel geometric shape made from chocolate) and a visually similar cue which was never eaten, following conditioning. A similar pattern of data was shown in Chapters 3 and 5, whereby a CS+, CS- and visually similar unseen objects captured attention to a greater extent than neutral distractors after single-trial conditioning. These data were interpreted as providing tentative evidence for generalisation of reward-driven attentional capture. This construal is consistent with prior work which found evidence that attention may also be captured by stimuli which share some defining feature with the original CS (Anderson et al., 2012). In their paradigm, participants learned probabilistic associations between coloured circles assigned either high or low monetary value. In a subsequent test phase, coloured flanker stimuli automatically captured attention when they were the same colour as the high-value stimuli presented in the conditioning phase. Thus, stimuli not previously paired with reward can automatically bias attention if they are perceptually similar to a reward-paired cue. However, the paradigm described by Anderson et al. (2012) is very artificial, laborious, involving simplistic stimuli and hypothetical monetary rewards. It remains to be determined whether stimulus generalisation can occur for more complex stimuli paired with primary reinforcers such as food.

Therefore, a second aim of this experiment was to explore whether attentional capture by food-paired cues generalises to other, similar stimuli using an adapted version of the emotional blink of attention task. As in previous experiments, responses were measured after just one learning trial. It was hypothesised that when stimulus discrimination was optimized, CS+ distractors would capture attention to a greater extent than CS- distractors. We also expected to see evidence of generalisation; i.e., that distractors that were visually similar to the CS+ would capture attention to a greater extent than those more similar to the CS-.

CS form: The role of stimulus novelty

An alternative explanation proposed for the ambiguous findings presented in chapters 3-5 relates to the impact of stimulus novelty on conditioned responses. The ability to acquire new information is essential for survival. The exploration of novel

objects can maximise new opportunities for learning, potentially enhancing the possibility of reward acquisition. This tendency to favour novelty can be seen in a phenomenon known as novelty bias, whereby a preference is shown for novel over known items. For example, in visual search tasks, attention has been reported to be preferentially allocated towards novel, rather than familiar distractors (Yang et al., 2009). In our previous experiments, the CS+ and CS- were abstract geometric objects made from marzipan/chocolate or plastic, respectively. The shapes were novel in appearance and designed to have neutral valence, so participants would have no prior associations which could interfere with conditioning. It is unclear what effect the mere novelty of an unusual experimental procedure and stimuli may also have had on responses. It is clear, however, that – despite more clear-cut effects on subjective measures of conditioning, the implicit EBA measure has been less effective in discerning the influences of reward learning and responses to novelty.

In typical human appetitive conditioning studies, researchers utilise simple, familiar visual stimuli such as a red circle or blue square as the CS. These shapes are then paired with a well-known food. Even in studies which have used novel CSs, the novel visual stimulus is presented separately from the US, so the food US is still highly familiar to participants (e.g., Armel et al., 2009). Therefore, the impact of CS+/US novelty in the specific, rather unusual paradigm employed in this thesis is currently unclear.

On the one hand, food is essential for survival, but a degree of caution when exploring novel foods would be highly protective. In support of this, Yamada, Kawabe & Ihaya (2012) demonstrated that food likeability decreases as categorization difficulty increases. The more uncertainty there was surrounding a potential food item's edibility, the less participants wanted to eat it. This avoidance of unusual or novel foods, therefore, may be an adaptive trait. Consequently, it seems plausible that a novel CS-US compound may be less liked and more resistant to conditioning than an object with a more familiar appearance.

Furthermore, novelty *per se* appears to be an important cue which can influence the salience of stimuli. Novel items involuntarily capture attention over familiar items when familiar and novel stimuli are equally salient (Horstmann & Herwig, 2015). In addition, Snyder, Blank and Marsolek (2008) demonstrated that when learned salience was induced via a classical conditioning procedure, there was competition for attention between learned cues and novel cues, as both were highly

salient. This may explain the lack of distinction between CS+ and CS- distractors through Chapters 3-5. Novel items may be equally salient as reward-paired cues, and thus capture attention to a similar extent, making it difficult to interpret results from the EBA task. A CS with a more familiar shape and appearance may be inherently less salient and therefore, more easily ignored.

Therefore, a third aim of the current study was to explore whether the degree of attentional capture by CS+ and CS- distractors was influenced by the novelty of the CS appearance. We expected to see a clear difference in attentional capture between familiarly-shaped CS+ and CS-, but less discrimination in the degree of capture when the CS shapes were novel.

Furthermore, evidence suggests that generalisation may also vary as a function of stimulus novelty and familiarity (Best & Batson, 1977). Those researchers noted that generalisation of conditioned aversion was reduced for familiar flavour stimuli to which rats were pre-exposed. However, strong generalisation occurred between a novel flavour CS and other novel flavours. This arguably suggests that familiar stimuli are more easily discriminable and, therefore, conditioning would not necessarily generalise to other stimuli even if they are perceptually similar. In the present context, the presentation of a range of unfamiliar, complex, abstract shapes which are less easy to discern may result in greater generalisation to a novel CS+.

Consequently, an additional aim of this experiment was to assess whether the relative novelty or familiarity of the CS objects would influence the degree of generalisation to similar stimuli. It was hypothesised that stronger generalisation would emerge when participants are assigned novel CSs, since the familiar CSs and generalisation stimuli would be more easily distinguishable.

Summary of aims and hypotheses

To summarize: the following sections report a new conditioning procedure designed to explore single-trial reward-learning, with specific examination of the effects of CS+ familiarity or novelty on subjective and attentional measures of conditioning, and generalisation stimuli that were visually similar to, or distinct from the CS+.

As evidenced by the findings of previous experiments and in line with the issues discussed above, the ability of the EBA to produce discernible effects attributable to increased salience of a CS+ through reward learning alone is

potentially confounded by the separate capacity of novelty *per se* to capture attention.

In relation to the specific aims of the present study, the following was anticipated:

- The emergence of a generalisation gradient after single-trial conditioning, such that subjective and attentional CRs are strongest for stimuli highly similar to a CS+ and decrease in intensity as stimuli become more distinct from a CS+.

- An innate tendency to attend to novel stimuli might mask detection of post-conditioning changes in EBA accuracy related solely to reward-related salience, and

- Less effective visual discrimination between related novel stimuli, than stimuli with a familiar appearance, leading to greater generalisation from a novel CS+ to similar stimuli.

6.3 Methods

6.3.1 Participants

Fifty-two participants completed the experiment (15 males). Participants were aged 18 – 40, had normal or corrected-to-normal vision and reported no use of medications known to affect vision, attention or appetite. Participants were screened prior to participation to ensure they liked white chocolate and had no food allergies or intolerances, using the same screening questionnaires described in previous chapters [See General Methods (Chapter 2)]. Participants were randomly allocated to a novel CS condition ($N = 26$) or a familiar CS condition ($N = 26$). All participants gave written informed consent and received £15 in vouchers, or course credit, for taking part. The study was approved by the University of Liverpool Ethics Committee.

6.3.2 Conditioning task

Two objects, differing in shape and colour, served as the appetitve cue (CS+) and neutral cue (CS-). The CS+ was an edible object made of chocolate, handmade to order by Choconchoc Ltd. The CS- was an inedible plastic object. In one condition, these objects were novel in appearance, and in the other they were familiar to participants. Shapes were selected on the basis of a pilot where participants were asked to rate a number of shapes for their perceived novelty and familiarity. Unlike in previous chapters, here the CS+ and CS- were produced in highly contrasting colours to maximise the ease of discrimination between them. The colour-shape assignment was counterbalanced across participants (see Table 6.1). Participants underwent just one conditioning trial, following the naturalistic conditioning procedure used in previous chapters (see Chapter 3). Participants were given two minutes to touch, handle and smell the CS- and to touch, handle, smell and taste the CS+. The CS+ and CS- presentation order was counterbalanced, and there was a rest period of approximately 90 seconds between each object presentation.

Table 6.1 Counterbalancing of colour-object assignment across each experimental condition.

	Familiar		Novel	
	Group 1	Group 2	Group 1	Group 2
CS+				
CS-				

6.3.3 Self-report measures of conditioning

6.3.3.1 Stimuli

Based on the procedure followed in similar studies (e.g., Haddad et al., 2013), six intermediate generalisation stimuli (GS) were created in order to test the degree of stimulus generalisation. The images of the CS+ and CS- were morphed together

using Adobe Illustrator to produce a series of generalisation stimuli varying incrementally in similarity between the CS+ and CS-. The 3D drawings of each GS were created in Google Sketch Up. Images were edited using Adobe Photoshop to ensure size and colour were matched between conditions. The stimuli are shown in Table 6.2.

Table 6.2 Conditioned stimuli and generalisation stimuli (GS) used in the explicit self-report task.

Familiar								
Novel								
Group 1	CS+	GS1	GS2	GS3	GS4	GS5	GS6	CS-
Group 2	CS-	GS6	GS5	GS4	GS3	GS2	GS1	CS+

CS+: conditioned stimulus paired with an appetitive outcome (chocolate ingestion) during conditioning; CS−: conditioned stimulus never paired with an appetitive outcome (handling plastic). Participants were randomly assigned to either a novel or familiar condition, referring to the novelty/familiarity of the CS appearance. In each condition, half the participants received a yellow CS+ and a purple CS-. This was reversed for the remaining participants. Based on the paradigm described by Haddad et al. (2013), 6 GSs were used to create a similarity gradient from the CS+ to the CS−. As shown, for each participant group GS1 refers to the generalisation stimulus most similar to the CS+.

6.3.3.2 Explicit self-report task

Based on previous experiments (e.g., Papachristou et al., 2013), self reported cravings, expectancies and liking were taken as explicit measures of conditioning and stimulus generalisation. See Table 6.3 for the wording of each question. Participants completed a short computer questionnaire comprising twenty four questions. Each of the eight object types were presented individually on the screen and participants were asked to rate them for the degree of cravings elicited, their US-expectancies and the object valence on 100 mm visual analogue scales (VAS). The order of question type and stimulus type were randomised. This task was completed once before and once after conditioning.

Table 6.3 Wording of questions and anchor points used on visual analogue scales presented during a self-report task where participants were required to rate the degree of pleasantness, cravings and expectancies elicited by the two CS and six GS stimuli.

Measure	Question	Anchors
Cravings	"When presented with this object, how strong is your craving for chocolate right now?"	"no craving at all" to "extremely strong craving".
US-Expectancy	"When presented with this object, how strongly do you now expect to be invited to eat chocolate?"	"certainly not" to "certainly".
Liking	"How pleasant do you find this object?"	"extremely unpleasant" to "extremely pleasant"

6.3.4 Emotional Blink of Attention

6.3.4.1 Stimuli

To reduce the length and complexity of the experiment, only the generalisation stimuli most similar to the CS+ and CS- were included as distractors (GS1 and GS6, respectively), termed below as GS+ and GS-. Models of the GS+ and GS- were created out of clay and coated in plastic to enable them to be photographed. Photographs were taken following the same process as in chapters 3-5. Fifty photographs of the CS+, CS-, GS+ and GS- were taken against a variety of neutral household backgrounds, to ensure image complexity was similar between these stimuli, and the other distractor, target and filler images, and to reduce contrast between image types. Images were resized to 320 x 240 pixels and matched for average luminosity using Adobe Photoshop. The same set of neutral images, rotated target images, and landscape filler images were used as in Chapters 3-5.

6.3.4.2 Task

The emotional blink of attention task (EBA) was adapted from that described in previous chapters (See Chapter 2 for a full task description) and followed a similar set up (for a schematic representation see Figure 6.1). Task instructions and stimuli presentation were kept consistent wherever possible. The only changes were to trial number and distractor categories. Distractors were always presented at lag 2 and were from one of five distractor categories: CS+, GS+, CS- GS- and neutral. Each

session had 160 trials in total, comprising 4 blocks of 40 trials. Distractors from each respective category were present on eight trials per block.

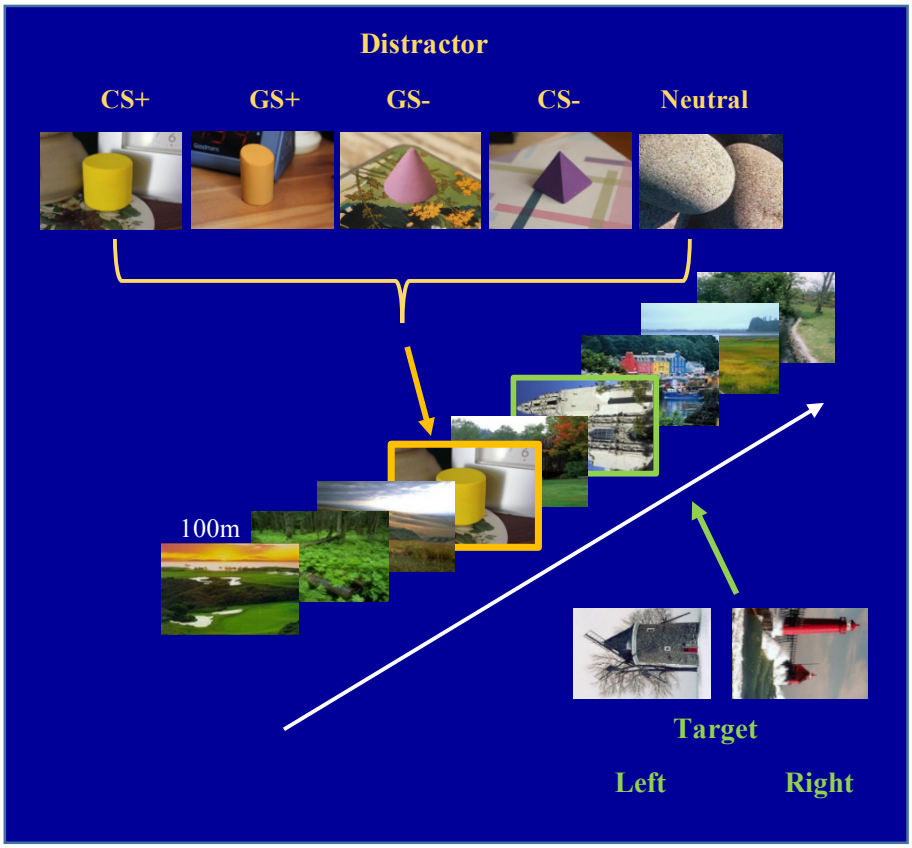


Figure 6.1 Diagram of EBA task, with example images from each distractor and target category.

6.4 Procedure

Participants responded via email to recruitment posters and online advertisement across the university campus and local area and invited to one experimental session in the University of Liverpool Psychology Department. Participants were asked to refrain from eating food and drinking calorie-containing beverages for at least two hours prior to arrival. Participants who completed the screening and were found to meet the eligibility criteria-completed the full experimental procedure (see Figure 6.2 for a summary), and were randomly assigned to either the novel or familiar condition. First, participants completed satiety ratings (full description in Chapter 2), immediately followed by the practice session EBA and task instructions, and the pre-conditioning EBA session. After this, participants completed the explicit VAS task,

rating each of the two CS and six GS stimuli for elicited cravings, expectancies and pleasantness. Participants were also asked to rate the familiarity of the CS+ and CS- objects on 100 mm VAS scales ranging from “extremely unfamiliar” to “extremely familiar”. All participants completed the conditioning procedure (see 6.3.2 for description). Following single-trial conditioning, participants completed a second set of hunger ratings, and repeated the EBA task and explicit self-report task. Participants rated the valence of a random sample of photographs used in the EBA task to ensure valence could not account for any differences in accuracy to certain distractors. Participants rated five images from each category of images including the landscape filler images; totalling thirty images overall. Participants then completed a short battery of questionnaires designed to measure aspects of personality and eating attitudes that may be related to single trial appetitive conditioning with chocolate as a US. These measures were taken to provide descriptive information about the sample and to ensure conditions were evenly matched on these constructs. To measure contingency awareness, participants were asked to recall the shape and colour of the CS+ and CS-. They also reported their perception of the experimental aims of the study. Finally, height and weight were recorded using calibrated scales and a stadiometer.-Participants received a full verbal and written debrief and were thanked for their participation.

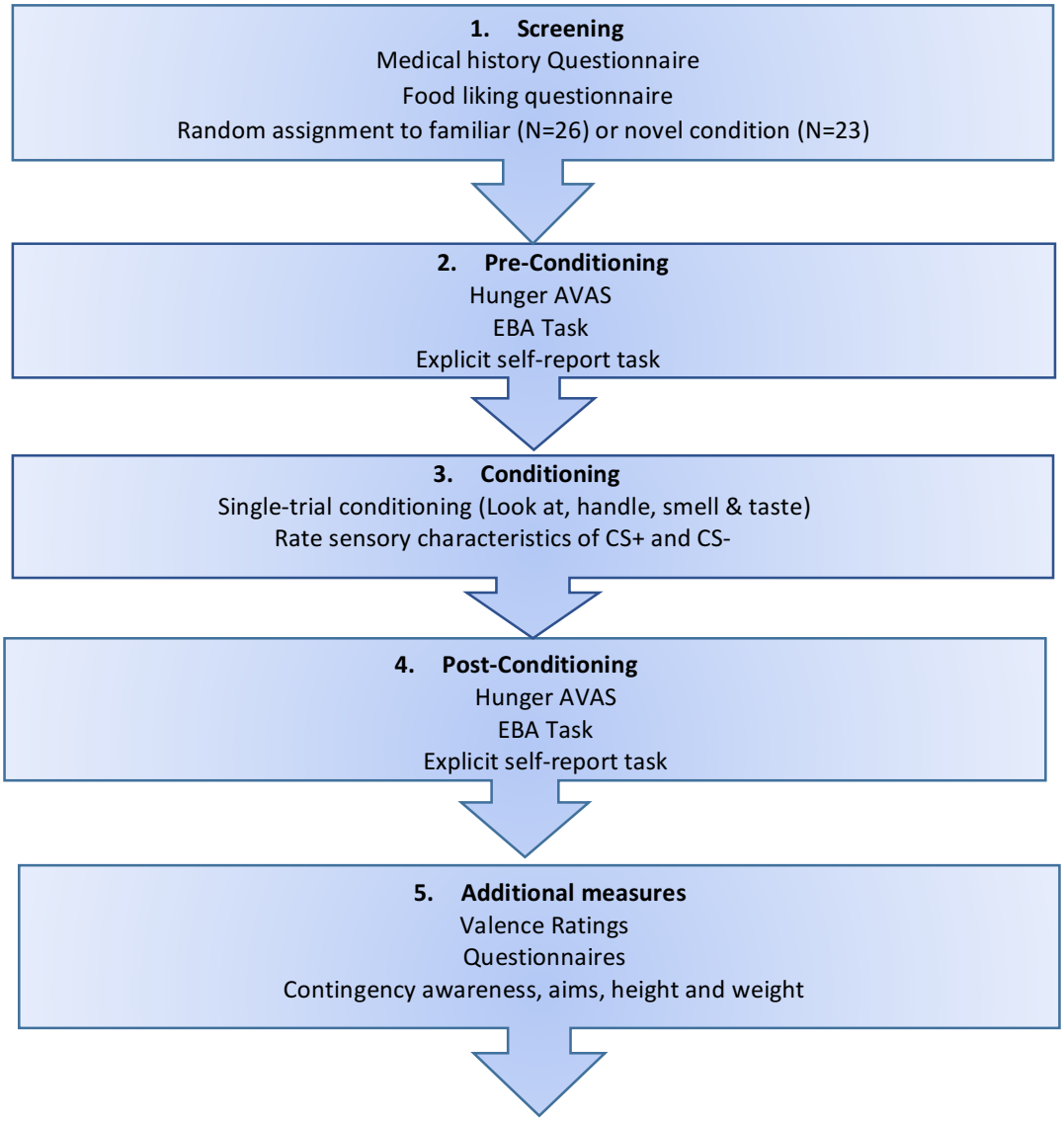


Figure 6.2 Flow chart depicting the experimental procedure.

6.5 Results

6.5.1 Pre-analysis checks and participant characteristics

Normality testing revealed that the EBA data was approximately normally distributed. Although a Shapiro-Wilks test highlighted some deviations from normality on self-report measures of conditioning, after assessing Q-Q plots and histograms these were deemed to be minor – in line with Ghasemi and Zahediasl (2012). As there is no suitable non- parametric equivalent to the tests reported here, and parametric tests are considered robust, analysis was conducted as per our original analysis plan. Where the assumption of sphericity was not met, Greenhouse-Geisser corrections were applied. *Post hoc* tests are reported with Bonferroni corrections for multiple comparisons.

Three participants with particularly poor performance on the EBA task were excluded from all analysis; their overall accuracy was below 25%, which is less than chance level. The remaining sample consisted of n=23 in the novel stimulus condition and n=26 in the familiar stimulus condition (see Table 6.4 for participant characteristics). On average participants were a healthy BMI (Mean ± SD = 23.53 ± 4.22 kg/m^2). The two conditions were well matched on participant characteristics, *Pillai's trace* = .24, $F(10, 38) = 1.21$, $p = .316$, $\eta_p^2 = .24$.

In terms of personality variables, levels of food neophobia were almost identical to that reported in a comparable sample of European university students (29.39 ± 10.07; Fenko, Leufkens & van Hoof, 2015). Eating attitudes were also consistent with mean scores of a sample of non-clinical Dutch undergraduates on DEBQ subscales restraint, emotional eating and external eating (2.47 ± 0.88; 2.48 ± 0.71; and 3.13 ± 0.51 respectively (van Strien, Peter Herman & Anschutz, 2011). BAS-RR scores were comparable to that of moderate scorers in a non-clinical sample (16.83 ± 2.26; Alloy, et al. 2006). The Brief-SSS: Total was also similar to that of a UK University based convenience sample (3.01 ± .59; Eachus, 2004)

Table 6.4 Participant characteristics for each condition. Values are means with standard deviations in parentheses.

	Familiar Stimulus (*n*=26)	**Novel Stimulus (*n*=23)**
Gender		
Female N (%)	18 (69%)	16 (70%)
Age	24.23 (5.07)	27.48 (5.20)
BMI	23.94 (5.31)	23.07 (2.55)
DEBQ		
Restraint	2.41 (.75)	2.57 (.64)
Emotional	2.76 (.93)	2.59 (.75)
External	3.57 (.58)	3.46 (.57)
FNS	29.31 (7.97)	28.22 (11.36)
BIS/BAS		
BAS-RR	17.27 (2.07)	16.22 (2.56)
Brief-SSS	3.08 (.63)	3.15 (.60)

BMI = body mass index, DEBQ = Dutch Eating Behaviour Questionnaire; FNS = Food Neophobia Scale; BIS/BAS = Behavioral Inhibition System (BIS) and the Behavioral Activation System (BAS); Brief-SSS = Brief Sensation Seeking Scale. BAS-RR = Behaviourl Activatiob System – Reward Responsivity. As the DEBQ has a distinct three-factor structure a total score was not computed.

No participant accurately guessed the aims of the experiment. Two participants referred to forming associations between shapes and chocolate but their exclusion did not alter results. All participants were aware of the CS-US contingencies.

6.5.2 Manipulation Check

The CS+ objects were rated as significantly more familiar in the familiar stimulus condition (Mean ± SD = 79.81 ± 19.52) than the novel stimulus condition (37.39 ± 27.67), $t(38.99) = 6.13$, $p < .001$, $g = 1.79$. Similarly, the CS- objects were rated as significantly more familiar in the familiar stimulus condition (79.15 ± 18.94) than the novel stimulus condition (26.61 ± 26.23), $t(47) = 8.10$, $p < .001$, $g = 2.32$.

6.5.3 Appetite

A 2 (time) × 2 (condition) mixed ANOVA revealed there were no main effects or interactions between time and condition on appetite ratings which remained moderately high across the experiment ($ps \geq .089$) (see Figure 6.3).

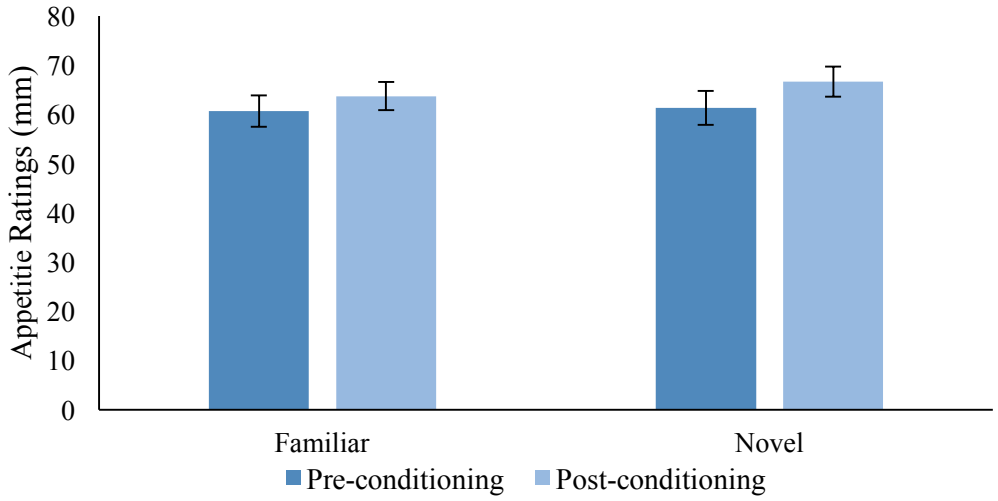


Figure 6.3 Mean appetite ratings pre- and post-conditioning for each experimental condition (familiar and novel). Bars represent standard error.

6.5.4 Conditioning

6.5.4.1 Taste ratings and Consumption

On average, participants found the taste of the CS+ very pleasant (73.78 ± 21.62). Taste ratings did not differ between the novel (76.43 ± 17.10) and familiar condition (71.42 ± 25.06) ($p = .424$). No significant outliers were identified. On average,

participants consumed 10.66 ± 1.36 g across the experiment (Min = 8.5 g, Max = 12.9 g) The amount consumed did differ slightly between conditions; chocolate consumption was slightly lower in the novel condition (9.93 ± .81) than in the familiar condition (11.30 ± 1.43), $t(40.45) = 4.19$, $p < .001$, $g = 1.16$. This very small difference is likely due to natural variation in product size and weight associated with the manufacturing process of the chocolate shapes. We do not anticipate such a small variation will influence results.

6.5.4.2 Conditioned Cravings

A 2 (condition) × 2 (time) × 8 (stimulus type) mixed ANOVA revealed a significant main effect of time, $F(1, 47) = 24.31$, $p = .001$, $\eta_p^2 = .34$, a significant main effect of type, $F(4.12, 193.72) = 5.97$, $p = .001$, $\eta_p^2 = .11$, and a time × stimulus type interaction, $F(4.78, 224.79) = 4.25$, p = .001, $\eta_p^2 = .08$. No significant main effects or interactions emerged with condition, so this factor was dropped from follow-up analysis.

Before conditioning there was no difference in the degree of self-reported cravings elicited by the CS+ or CS-, $t(48) = 1.42$, p = .163, $d = .20$. However, post-conditioning the CS+ elicited significantly greater cravings than the CS-, $t(48) = 5.71$, p < .001, $d = .82$, indicating that differential appetitive conditioning was successful in a single-trial.

Prior to conditioning, self-reported cravings for chocolate were similar in response to each of the six generalisation stimuli, as well as the CS+ and CS- ($ps \geq 1.00$). However, post-conditioning, differences emerged between stimulus types, $F(3.83, 183.93) = 8.66$, $p = .001$, $\eta_p^2 = .15$, with a significant linear trend, $F(1, 48) = 20.62$, $p = .001$, $\eta_p^2 = .300$, suggesting that the degree of generalisation decreased as stimuli became less similar to the CS+.

In order to identify precisely which stimuli elicited generalised cravings post-conditioning, seven simple contrasts were conducted using the CS− as the reference condition. This revealed significant differences in self-reported cravings between the CS− and GS1, $F(1, 48) = 6.72$, $p = .013$, $\eta_p^2 = .12$. There was also a tendency towards significant differences between the CS− and GS class 2, $F(1, 48) = 3.78$, $p = .058$, $\eta_p^2 = .073$, but not between the CS− and GS 3 – 6 (see Figure 6.4), suggesting that self-reported cravings generalised from the CS+ to the most similar GS stimuli.

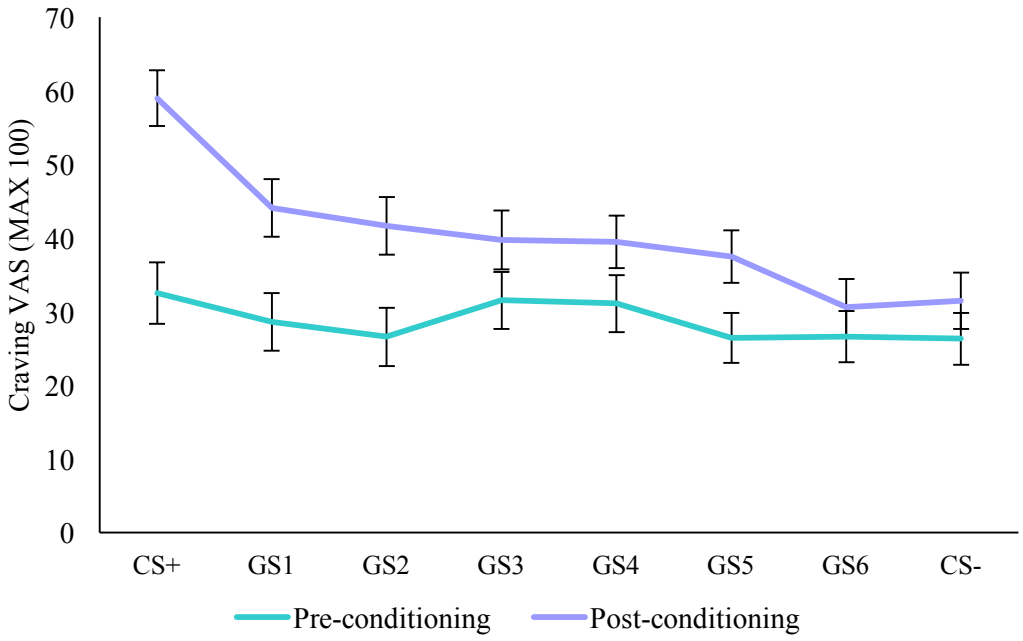


Figure 6.4 Average self-report conditioned cravings to a CS+, CS- and six generalisation stimuli, both pre- and post-conditioning.. Error bars reflect the standard error of the mean. CS+ = conditioned stimulus paired with chocolate; CS- = conditioned stimulus paired with no reward (plastic); GS1-6 = generalisation stimuli with GS1 being most similar to the CS+ and GS6 the least similar.

6.5.4.3 Conditioned Expectancies

A 2 (condition) × 2 (time) × 8 (stimulus type) mixed ANOVA revealed a significant main effect of time, $F(1, 47) = 22.72$, $p = .001$, $\eta_p^2 = .33$, a significant main effect of type, $F(4.45, 209.06) = 8.90$, $p = .001$, $\eta_p^2 = .16$, and a time × stimulus type interaction, $F(4.82, 226.64) = 6.41$, $p = .001$, $\eta_p^2 = .12$ on conditioned expectancies for chocolate. No significant main effects or interactions emerged with condition, so again this factor was dropped from follow-up analysis.

Post-conditioning the CS+ elicited significantly greater expectancies than the CS-, $t(48) = 6.82$, $p < .001$, $d = .97$. Whilst the CS+ did elicit slightly larger expectancies for chocolate than the CS- before conditioning, this was a much smaller magnitude and likely a chance finding as colour and shape were counterbalanced, $t(48) = 2.44$ $p < .018$, $d = .35$. Consequently, it appears conditioning was successful.

Pre-conditioning, expectancies for chocolate did differ by stimulus type, $F(5.50, 264.14) = 3.02$, $p = .009$, $\eta_p^2 = .06$, but polynomial contrasts revealed no meaningful trend.

As anticipated, the simple effect of stimulus type was significant, $F(4.53, 217.65) = 12.24$, $p = .001$, $\eta_p^2 = .20$, with a significant linear trend post-conditioning, $F(1, 48) = 31.91$, $p = .001$, $\eta_p^2 = .40$, suggesting that the degree of generalisation decreased as stimuli became less similar to the CS+.

In order to identify precisely which stimuli elicited generalised expectancies, seven simple contrasts were conducted using the CS− as the reference condition. This revealed significant differences in self-reported expectancies between the CS− and GS1, $F(1, 48) = 10.89$, $p = .002$, $\eta_p^2 = .19$, CS- and GS2, $F(1, 48) = 4.19$, $p = .046$, $\eta_p^2 = .08$, CS- and GS4, $F(1, 48) = 4.50$, $p = .039$, $\eta_p^2 = .09$, and a marginally significant difference between CS- and GS3 $F(1, 48) = 3.83$, $p = .056$, $\eta_p^2 = .07$. No significant differences emerged between the CS- and GS5 or GS6 ($ps > .144$) (see figure 6.5), suggesting that self-reported expectancies generalised from the CS+ to the most similar GS stimuli.

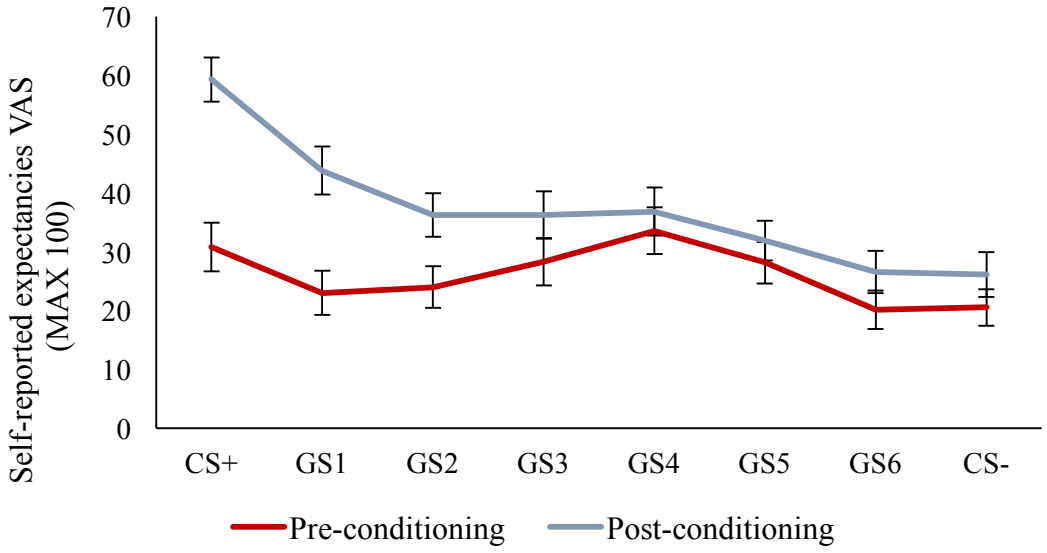


Figure 6.5 Average self-report US-expectancies to a CS+, CS- and six generalisation stimuli both pre- and post-conditioning. Error bars reflect the standard error of the mean. CS+ = conditioned stimulus paired with chocolate; CS- = conditioned stimulus paired with no reward (plastic); GS1-6 = generalisation stimuli with GS1 being most similar to the CS+ and GS6 the least similar.

6.5.4.4 Conditioned Liking

A 2 (condition) × 2 (time) × 8 (stimulus type) mixed ANOVA revealed a significant main effect of type, $F(3.56, 167.38) = 3.85$, $p = .007$, $\eta_p^2 = .08$. The main effect of time and the time type interaction failed to reach significance ($ps > .136$). No

significant main effects or interactions emerged with condition (*p*s > .259), so this factor was dropped from follow-up analysis.

Before conditioning there was no difference in the degree of self-reported likings for the CS+ or CS-, $t(48) = 1.01$, $p = .317$, $d = .14$. However, post-conditioning the CS+ was liked significantly more than the CS- , $t(48) = 2.17$, $p = .035$, $d = .31$, suggestive of successful differential appetitive conditioning.

Despite the lack of a significant time × type interaction, in line with our original aims, further follow up analysis was focused on the post-conditioning data to explore the generalisation gradient. This revealed a significant quadratic trend, $F(1, 48) = 9.99$, $p = .003$, $\eta_p^2 = .17$, suggesting liking decreased as GS stimuli decreased in similarity to the CS+ and began to increase again as they increased in similarity to the CS-. Each of the six generalisation stimuli was liked to a similar extent as the CS- (*p*s >.062) (see figure 6.6).

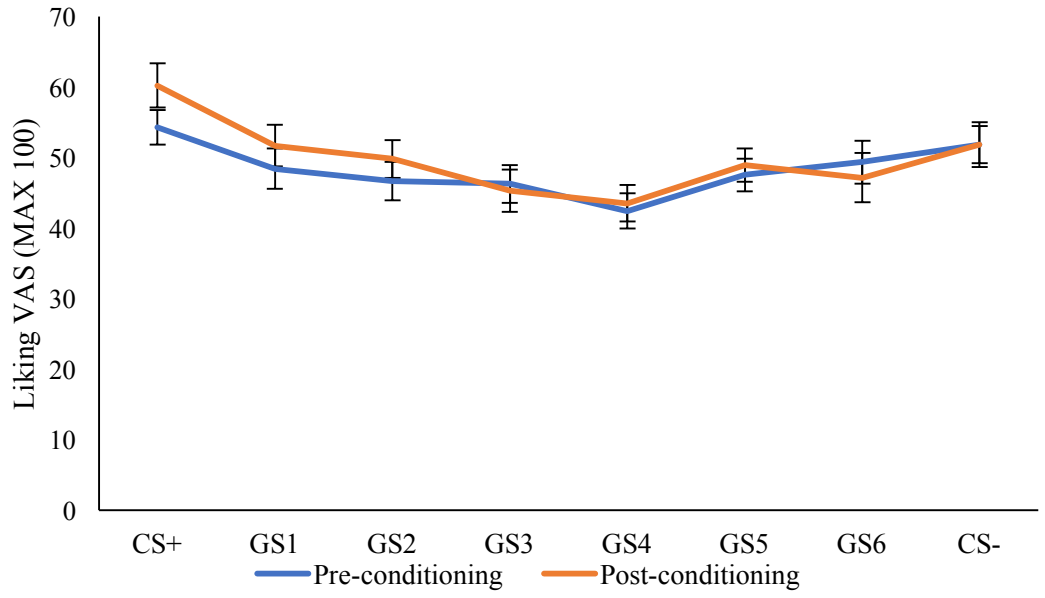


Figure 6.6 Average self-report liking of a CS+, CS- and six generalisation stimuli both pre- and post-conditioning. Error bars reflect the standard error of the mean. CS+ = conditioned stimulus paired with chocolate; CS- = conditioned stimulus paired with no reward (plastic); GS1-6 = generalisation stimuli with GS1 being most similar to the CS+ and GS6 the least similar.

6.5.5 EBA

6.5.5.1 % Accuracy

A 2 (condition) × 2 (time) × 5 (distractor type) mixed ANOVA revealed a significant main effect of time and type, as well as a time × type interaction on the percentage of correct responses made on the EBA task, $F(4, 188) = 6.46$, $p = .001$, $\eta_p^2 = .12$. No other main effects or interactions were significant. Consequently, condition was dropped for all subsequent analyses (See Appendix F for supplementary analyses).

A series of paired t-tests revealed that accuracy significantly decreased for all distractor types, except neutral distractors (see Table 6.5 Figure 6.7), suggestive of acquired salience across the conditioning procedure for all conditioned and generalisation stimuli.

Table 6.5 Comparison of pre- and post-conditioning target detection accuracy by distractor type (% correct responses) in the EBA task across both experimental conditions.

Distractor type	Mean ± *SD*		Statistics	
	Pre-conditioning	Post-conditioning	t-value	*p-value*
CS+	73.15 (*13.77*)	62.76 (*15.83*)	6.41	< .001
GS+	69.58 (*15.02*)	59.89 (*17.24*)	5.72	< .001
CS-	71.81 (*13.72*)	62.69 (*15.98*)	4.81	< .001
GS-	68.30 (*14.96*)	61.99 (*16.75*)	2.97	.005
Neutral	72.45 (*12.88*)	72.19 (*12.81*)	.18	.860

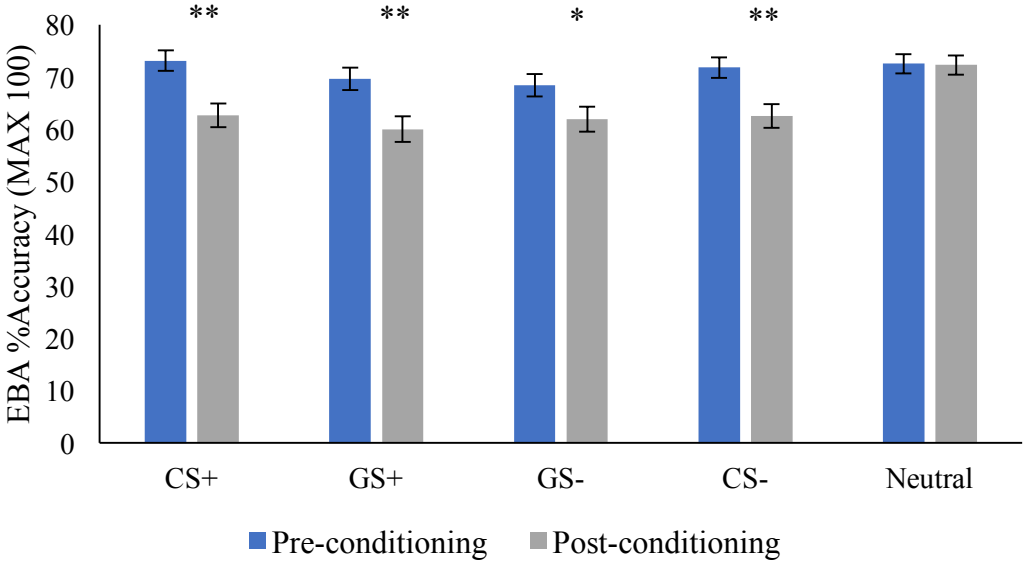


Figure 6.7 Mean target detection accuracy on an EBA task before and after naturalistic single-trial conditioning. Bars represent standard error. *p<.05, **p<.01, ***p<.001.

Contrary to predictions, accuracy for the five distractor types was unequal prior to conditioning, $F(4, 192) = 3.25$, $p = .019$, $\eta_p^2 = .06$. Accuracy for the GS- distractors was slightly lower than for the CS+ distractors ($p = .02$). As colour and shape were counterbalanced, and the objects had no conditioned meaning to participants at this stage, this is most likely a chance finding.

Post-conditioning, there was again a significant difference between distractor types on accuracy, $F(4, 192) = 12.53$, $p = .001$, $\eta_p^2 = .21$. Each of the four objects (CS+, GS+, CS- and GS-) were significantly more distracting than neutral distractors (ps < .001).

6.5.5.2 Reaction times

A 2 (condition) × 2 (time) × 5 (distractor type) mixed ANOVA revealed a significant main effect of time, $F(1, 47) = 26.53$, $p = .001$, $\eta_p^2 = .36$. Reaction times post-conditioning (236.39 ± 90.82) were significantly faster than pre-conditioning (255.24 ± 83.01). No other main effects or interactions were significant (ps >.082)

6.5.5.3 Valence ratings (VAS)

A 2 (condition) × 6 (image type) mixed ANOVA revealed a main effect of image type, $F(3.64, 171.15) = 23.20$, $p = .001$, $\eta_p^2 = .33$, a main effect of condition, $F(1, 47) = 6.35$, $p = .015$, $\eta_p^2 = .12$ and a condition x image type interaction, $F(3.64, 171.15) = 2.66$, $p = .039$, $\eta_p^2 = .05$ (see figure 6.8). Overall, the landscape filler images received higher valence ratings than all other image types, $ps < .001$. Participants in the novel condition gave the CS+ photographs used throughout the EBA task higher valence ratings than those in the familiar condition, $t(47) = 3.56$, $p = .001$, $g = 1.02$.

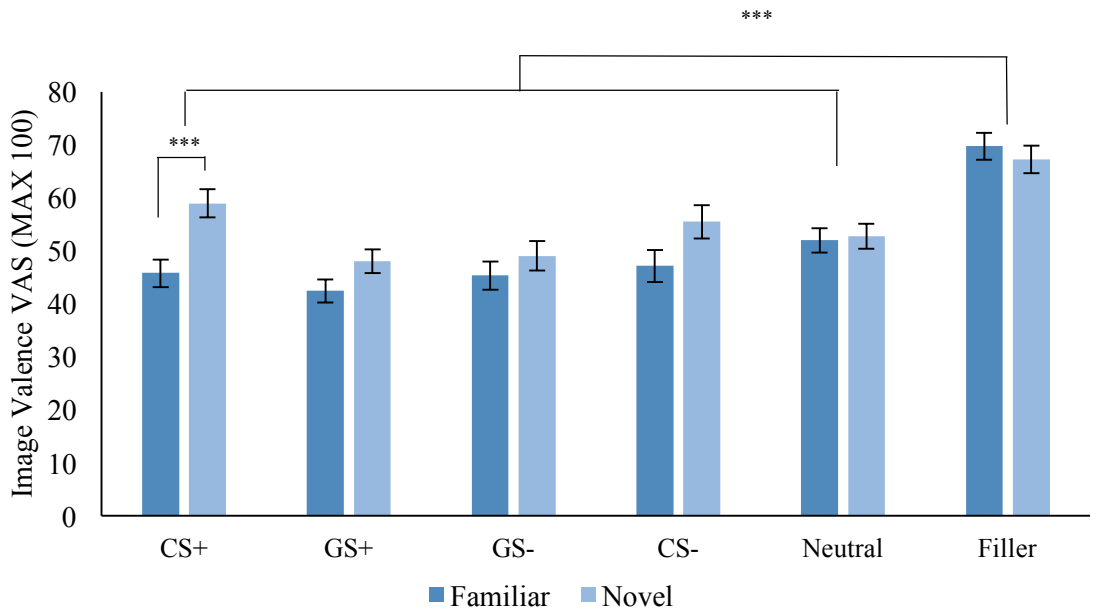


Figure 6.8 Mean valence ratings of a random sample of images from the EBA task, measured on 100 mm VAS, both before and after conditioning. Bars represent standard error. *p<.05, **p<.01, ***p<.001.

6.5.6 Associations with individual differences

Correlations were conducted between individual differences (e.g., eating attitudes, incentive sensitivity) and key outcome measures; difference scores (post-conditioning – pre-conditioning) for self-report measures (craving, US-expectancy and liking) and difference scores (post-pre) for attentional capture (% accuracy on EBA task). After applying corrections, one correlation remained significant. However, inspection of the data revealed that this associations was driven by an

outlier. Consequently, this association is not discussed further. (See Table F.2, Appendix F for correlation matrix).

6.6 Discussion

This study investigated the acquisition of conditioned appetitive responses to a novel or familiarly-shaped food cue (a 3D object made of chocolate) within a single conditioning trial. Of particular interest was how CRs to a specific food cue could then generalise to visually similar, neutral cues which had not been paired with the taste of chocolate. Overall, the current findings provide evidence of differential appetitive conditioning on self-report measures of conditioned cravings and expectancies. After just one conditioning trial in which participants ate the CS+ (a chocolate object) and handled the CS- (a plastic object), images of the CS+, but not the CS-, elicited strong conditioned cravings and expectancies for chocolate. Furthermore, these conditioned responses generalised to the stimuli closest in similarity to the CS+. Stimuli which were most visually similar to the CS+, in terms of shape and colour, elicited greater expectancies than dissimilar stimuli regarding chocolate expectancy, and greater cravings for chocolate. Self-reported liking was less clear; whilst the CS+, but not CS-, was rated as better liked after conditioning, there was no evidence that liking generalised to other visually similar stimuli.

This study builds on prior research on fear conditioning using generalisation stimuli derived by incremental morphing between distinct CS+ and CS- (Lisseck et al, 2008; Haddad et al., 2013), which showed evidence of robust generalisation gradients. To the best of our knowledge, the present study is the first example of such a paradigm being successfully adapted for use with an appetitive CS+, and provides the first demonstration that generalisation gradients occur with appetitive stimuli in human adults. Even more compelling is the fact that these effects appeared after just one learning trial, indexing how fast and, perhaps implicit, this process is.

This finding has important implications in relation to human eating behaviour. It is well established that Pavlovian conditioning processes play an important role in food cue reactivity (van den Akker, Schyns & Jansen, 2018). Theoretically, any cue can become associated with palatable food intake (e.g., sight of food, emotional states, satiety; Jansen, 1998) and elicit conditioned physiological (e.g., skin conductance response; Andreatta & Pauli, 2015), psychological (e.g.,

cravings; Papachristou et al., 2013), and behavioural responses (e.g. approach tendencies; Wardle, Lopez-Gamundi & Flagel, 2018). Thus, such effects may also extend to perceptually similar stimuli; stimuli which have never been associated with a US could elicit CRs if they share some similarity to a CS+. Given that this generalisation was apparent here after just one learning trial, it is clear that the range of potential stimuli in the real world capable of eliciting conditioned responses related to the desire for, and acquisition of, palatable food is both vast and rapidly expanding. Further research will be important for determining exactly how the processes uncovered here might influence eating behaviour and food choice in the real world.

Data from the attention task were less clear. Participants completed an EBA task before and after conditioning. The CS+, CS- and two generalisation stimuli with similarity to the CS objects (GS+ and GS-) served as distractors in the EBA, alongside neutral images. A significant drop in accuracy was observed for all but the neutral distractors from pre- to post-conditioning, with no differences emerging between distractor categories. It is unclear from the present data whether this truly represents a case of stimulus generalisation; it was anticipated that CS- stimuli would capture attention to a lesser degree than CS+ stimuli. However, it may suggest that salience acquired throughout naturalistic appetitive conditioning can spread from a CS+ to other visually similar stimuli.

It was also hypothesised that when CS appearance was familiar (classical geometric shapes), rather than novel (abstract and unique shapes), it would be easier to distinguish the CS+ from the CS-, thus facilitating differential responding and limiting any generalisation (Best & Batson, 1977). However, the present data failed to support this prediction. Instead, no differences emerged between the familiar and novel conditions on either the explicit self-report measures of conditioning, or the implicit EBA responses.

Work by Honey (1990), may elucidate why the results were not as anticipated. When rats were trained with test stimuli which were matched in terms of their novelty (i.e., both were familiar or both were novel), generalisation was stronger than when the stimuli differed in their familiarity (i.e., one was novel and the other was familiar). This seems to suggest that familiarity and novelty act as standard elements in stimulus representation (Robinson, Whitt & Jones, 2017). Whilst it is well known that perceptual similarity is important for stimulus

generalisation, there is growing evidence to support the view that *conceptual* similarity enhances stimulus generalisation (Dunsmoor, White & LaBar, 2011). Therefore, it seems that rather than novelty and familiarity differentially influencing conditioning and generalisation, they instead act as abstract concepts by which generalisation may spread (i.e., conditioned responses from a novel CS may spread to other novel stimuli, and also for similar familiar stimuli), perhaps accounting for the equal salience across all distractors. It may be useful in future work to explore effects when the CS+ and CS- differ conceptually or categorically (e.g., a novel CS+ and familiar CS-), as well as perceptually.

Based on this experiment, it seems that the appearance of the CS+ and CS- in terms of degree of novelty, shape and colour makes no real difference to the conditioning procedure, reinforcing the view that any cue can realistically become paired with reward even after just one learning trial.

Our experimental manipulation rests on the assumption that the object's appearance (colour-shape combination) determined its novelty or familiarity. Whilst self-report ratings of familiarity suggest our manipulation was successful, it is worth noting that novelty is a complex concept and could come from several sources. For example, unusual scenes which violate expectations appear to rapidly capture attention (Becker, Pashler & Lubin, 2007). Thus, an image of a vehicle parked on its front end drew attention quicker, and was fixated on for longer than a vehicle parked normally. This raises the possibility that the unusual nature of the conditioning procedure, rather than the visual appearance of the shapes introduced a potential source of conflict throughout the EBA task.

During the procedure, participants are asked to look at, smell and handle the CS- and look at, handle, smell and eat the CS+. Prior to the experiment participants have not had any experience with these particular objects, so it seems likely that the conditioning will violate any expectations about the object properties. The unexpected nature of the conditioning procedure may, therefore, be responsible for the increase in attentional capture by each object category. This explanation seems consistent with our prior experiments which have also found that both CS+ and CS- reliably capture attention to a greater extent than neutral distractors following our conditioning procedure.

In support of this explanation, Foley, Jangraw, Peck and Gottlieb (2014) found evidence that novelty can enhance salience independently of reward learning.

The authors argue that even after learning the reward value of a novel stimulus, it could be beneficial to continue attending to cues predicting negative or neutral outcomes to settle any remaining uncertainty about the reward outcomes, or to allow further processing of its visual attributes to commit to working memory. In our particular paradigm, it makes sense that attention would still be drawn to objects that could theoretically signal food availability, even if the possibility is remote. Learning to ignore neutral stimuli after just one learning episode could be costly if advantageous opportunities are later missed. Future research is warranted to better understand the links between novelty, reward and attention, and to understand how they might impact on subsequent behaviour.

It is also worth considering the possibility that, as images were presented rapidly (100 ms each), and all four object types were visually very similar, it is possible that participants were prevented from accurately distinguishing between the conditioned stimuli and generalisation stimuli. However, in other EBA or attentional blink paradigms, participants have been shown to automatically distinguish between highly complex, and often perceptually similar cues even when images are presented very rapidly. For example, familiar faces capture attention to a greater extent than unfamiliar faces when presented as distractors at lag-2 (Gobbini et al., 2013). Even when images are presented for just 70 ms, participants are able to accurately report the gender of a face presented in an RSVP stream (Müsch, Engel & Schneider, 2012).

A final point for consideration is that the technique used in this experiment to create the generalisation stimuli may not have produced completely equal steps. The CS+ and CS- were morphed together to create six intermediate steps using a combination of media editing software. The resulting images were consequently an approximation. Thus, the difference in similarity between the CS+ and CS- may not have been consistent between each image, unlike Haddad et al. (2013) who used software specifically designed to morph photographs of faces in equal stages. Nonetheless, conditioned cravings and expectancies elicited by the CS+ resulted in a generalisation gradient similar to that expected.

In summary, we present a novel paradigm which can assess single trial appetitive conditioning and stimulus generalisation. To our knowledge, this is the first paradigm to show generalisation gradients with an appetitive food CS in humans. This paradigm is easy and quick to implement, and the fact that the CS and

US form a compound leads to robust conditioned responses after just one conditioning trial. This paradigm may prove important for further understanding the role of conditioning in overeating, and investigating individual differences in reward learning. Whilst effects are clear for explicit measures of conditioning, changes in implicit attention are unclear; both the CS+, CS- and generalisation stimuli reliably and automatically captured attention post-conditioning. We argue that events that violate expectations and are consequently imbued with enhanced salience should be studied in more detail. Stimuli for which the outcome is uncertain may automatically capture attention even though participants are aware that associations with reward are weak. Therefore, this paradigm warrants further investigation to better understand how stimulus generalisation may impact upon human eating behaviour, and to explore how knowledge and expectations about a stimulus can influence salience, perhaps independently of reward.

Chapter 7

Neural correlates of single-trial appetitive conditioning.

7.1 Abstract

Earlier work in this thesis suggests that naturalistic single-trial appetitive conditioning is a putatively potent phenomenon in humans, capable of modulating both motivation and attention. At present, the neural underpinnings of such rapid learning are unknown. We explored brain activation underlying single-trial conditioning with functional magnetic resonance imaging (fMRI). Twenty-three healthy adults (12 males) underwent conditioning during which they ate a novel 3D object made from chocolate (CS+) and handled a similar object made from plastic (CS-). Brain activity was recorded before and after conditioning during a passive viewing paradigm. In addition, fMRI was used post-conditioning to explore activity when distractors differing in their reward associations (CS+, CS- or neutral) served as distractors in an emotional blink of attention task (EBA). Regarding subjective ratings, the CS+ was rated as more highly craved, better liked and elicited greater expectancies for chocolate than the CS− after conditioning. An exploration of the interaction between time (pre- and post-conditioning) and CS type (CS+, CS-) during the passive viewing task revealed tentative evidence of enhanced activation from pre- to post-conditioning in the right superior frontal gyrus (R.SFG) in response to the CS-. Behavioural performance on the EBA task was unaffected by distractor type. However, brain regions related to reward and attention were activated more strongly by CS+ distractors than CS- distractors (insula and cuneus) or neutral distractors (superior and medial temporal gyrus). The results reveal neural correlates of single-trial appetitive conditioning in humans for the first time, and make a novel contribution by highlighting a role of response inhibition during learning about non-rewards, perhaps optimising motivated behaviour.

7.2 Introduction

A combination of animal and human studies has begun to elucidate the neural correlates of appetitive conditioning and have highlighted a complex neural network including the amygdala, orbitofrontal cortex (OFC), anterior cingulate cortex (ACC) and striatum (Martin-Soelch et al., 2007). The amygdala has been reliably shown to play a key role in the attribution of emotional significance to different events, and appears to represent the value of both appetitive and aversive outcomes learned during Pavlovian conditioning (Paton, Belova, Morrison & Salzman, 2006). Enhanced neuronal plasticity has been observed in the amygdala as a result of stimulus-response associations (Uwano, Nishijo, Ono & Tamura, 1995). In particular, the basolateral amygdala (BLA) appears to encode the sensory-specific features of a particular outcome associated with reward, whilst the central nuclei (CeN) has been implicated in more general affective and motivational processing (Balleine & Kilcross, 2006).

Neuroimaging research in humans has generally found the orbitofrontal cortex (OFC) to be involved in appetitive conditioning processes (e.g., Gottfried O'Doherty & Dolan, 2002; Cox, Andrade & Johnsrude, 2005), and it is thought to encode the expected outcome associated with a particular rewarding stimulus (Schoenbaum Chiba & Gallagher, 1998; Roesch & Olson, 2004). Through connections with the BLA, the OFC may receive information about the incentive value of a predictive cue, thus guiding behaviour and decision making (Schoenbaum, Setlow, Saddoris & Gallagher, 2003).

The ACC may be activated in response to the anticipation of reward (Kirsch et al., 2003) and is thought to be primarily involved in discriminating between stimuli based on their reward associations (Cardinal et al., 2003). The striatum, and particularly the nucleus accumbens (NAc), is thought to play a key role in cue-elicited approach-motivated behaviours in appetitive conditioning (Day & Carelli, 2007). In line with an 'actor-critic' model (O'Doherty et al., 2004), the dorsal striatum (the 'actor') is heavily implicated in instrumental learning, and has projections to motor areas, modulating behavioural action (Chase et al., 2015). Conversely, the ventral striatum, (the 'critic') is implicated more heavily in Pavlovian association learning. It appears to receive information from the ACC and encode the expected and actual reward outcomes to generate prediction errors (van der Meer & Redish, 2011).

Traditionally, attention has been viewed as a dual-process: current goal set modulates a dorsal frontal-parietal neural network via top-down attentional control, whilst a ventral fronto-temporal parietal network, including the temporo-parietal junction (TPJ) controls stimulus-driven attention in a bottom-up fashion (Corbetta & Shulman, 2002). However, research has begun to uncover a third neural network which governs value-driven attentional capture, comprising the visual corticostriatal loop and the intraparietal sulcus (IPS; Anderson, 2017). The corticostriatal loop incorporates the early visual cortex, caudate tail and lateral occipital complex (LOC). The primary visual cortex appears to be important for coding the value of a stimulus (Itthipuripat, Vo, Sprague & Serences, 2019). Furthermore, evidence from ERPs demonstrates modulation of an early neural response, P1, by previously rewarded stimuli even when they are task irrelevant and physically non-salient (MacLean & Giesbrecht, 2015), suggesting a role of the extrastriate cortex in value-driven attention (Anderson, Laurent & Yantis, 2014).

The caudate tail shares connections with the extrastriate cortex and recent evidence shows that this network assigns attentional priority to stimuli based on their associations with reward (Anderson et al., 2014), particularly for stimuli whose value is relatively stable. By contrast, the caudate head encodes the value of objects whose values are uncertain or which fluctuate (Kim & Hikosaka, 2013).

Furthermore, the object-specific lateral occipital complex appears to be consistently activated by reward-associated stimuli and is also heavily involved in object-recognition for both novel and familiar items (Margalit et al., 2016). Finally, the parietal cortex has also been implicated in reward-driven attention. The IPS generates a spatial priority map which combines signals about stimulus value, physical salience and goal-set; activation may reflect competition between sources of salience (Anderson, 2017).

There are a wide variety of tasks and technologies employed to measure visual attentional processing, many of which focus on spatial attention (Carrasco, 2011). However, more recently, research has focused on the temporal processing of attention and its capacity limits, particularly for emotionally salient stimuli. The emotional blink of attention task (EBA) is one such task, which provides a measure of stimulus salience. In the standard EBA task, attention is consumed by the processing of a distractor which has some affective or motivational significance,

leaving attention unavailable for the next 200-500 ms, subsequently impairing processing of a target (Most et al., 2005).

Whilst the majority of research has focused on the ability of highly affective stimuli to induce a 'blink' (e.g., Most et al., 2007), several studies have shown the power of motivationally salient stimuli – such as food during hunger, to capture attention (Piech et al., 2010). Furthermore, the EBA appears to be sensitive to fluctuations in stimulus value: neutral stimuli acquire the ability to generate a 'blink' after they acquire salience through an aversive Pavlovian conditioning paradigm (Smith et al., 2006). The data presented throughout Chapters 3-6 suggest that these modulations may be modest, yet – due to the potential utility of this task – it is important to attempt to build a fuller understanding of the underlying mechanisms. Currently, very little is known about the neural substrates of the EBA (McHugo, Olatunji & Zald, 2013).

The amygdala has received the most attention in this regard, due to its well-known role in processing affective stimuli (Anderson & Phelps, 2001; Davis & Whalen, 2001). Most et al. (2006) found that during an EBA task, emotional distractors were associated with greater amygdala activation. However, for those instructed to maintain a specific attentional set, rostral anterior cingulate cortex (ACC) activity increased alongside a decrease in amygdala activation, suggesting a possible role for the rostral ACC in attempting to inhibit attentional capture by distracting stimuli. However, in that experiment, neural activity was only recorded for trials where no target was present, making it difficult to determine whether similar mechanisms underlie a true 'blink' in an EBA paradigm.

Schwabe et al. (2011) conducted an fMRI experiment using a dual-target attentional blink task, similar in many ways to the EBA. In their paradigm, participants had to detect an emotional first target (T1) as well as a second neutral target (T2). Attentional capture by an emotional T1 was associated with a long 'blink' and activity in a ventral attention network (VN) comprising the insula, OFC and ACC, suggesting a potential mechanism for how motivationally salient stimuli may steer attention in a 'bottom-up' fashion.

As well as a distinct lack of evidence regarding the neural substrates of the EBA in general, currently no studies have attempted to elucidate the neural mechanisms underlying value-driven attentional capture in the EBA paradigm.

Consequently, the present study aimed to build on the existing literature and address some of these issues.

Firstly, we aimed to measure subjective conditioned responses acquired through a naturalistic single-trial conditioning procedure, at a behavioural level. The visual appearance of a neutral 3D geometric object was conditioned to a sweet taste through consumption in a single conditioning episode: participants were asked to eat the object, made of white chocolate (CS+). Participants handled a second similar object, of a different shape and colour, made of plastic (CS-). It was predicted that subjective markers of conditioning (craving, expectancy and liking) would increase for the CS+ but not the CS- from pre- to post-conditioning.

A second aim was to investigate neural correlates of single-trial naturalistic appetitive conditioning in humans, with a specific interest in neural correlates associated with attentional capture by task-irrelevant cues differing in their reward history. Thus, CS+, CS- and neutral distractors were presented rapidly in a single-target EBA task whilst neural activity was recorded.

Although fMRI offers excellent spatial resolution, the brain's hemodynamic response time does limit its temporal resolution. Neural processes happen rapidly and occur continuously, yet the blood-oxygen-level dependent signal (BOLD) takes approximately 6 s to reach its peak (Glover, 2011), then only returns to baseline approximately 15 s after the initial stimulus onset (Brühl, 2015). Although modern analysis methods, and methodological techniques such as jittering event-related stimuli mean that BOLD signals at shorter intervals (~100 ms) can be accurately captured (Ogawa et al., 2000), we considered it optimal to combine both response dependent (EBA) and passive viewing tasks in an attempt to overcome such limitations.

Furthermore, overcoming these temporal limitations involves the addition of prolonged inter-stimulus intervals (ITIs) which significantly increases task length, and due to the cognitive and motor demands associated with the task, practice effects may be problematic. Consequently, the EBA task was only completed in the scanner once, only after conditioning. Conversely, passive viewing paradigms involve participants simply viewing experimental stimuli with no response requirement. Crucially, they are free from practice effects, which was particularly important in our pre- / post-conditioning design, and they are unaffected by motor responses and task demands (Goodyear, Liebenthal & Mosher, 2014). Consequently, images of the CS+

and a CS- were presented in a passive viewing paradigm before and after conditioning while neural activity was recorded using fMRI. In addition, we administered the EBA task, which involved both the rapid presentation of stimuli, and introduced additional task demands due to the requirement of making responses on each trial.

We hypothesised that after just one conditioning trial, passive viewing of the CS+ would be associated with greater activity in reward-related areas such as the amygdala, ventral striatum and orbitofrontal cortex, relative to the CS-. In addition, neural activity was recorded whilst participants underwent an EBA task, post-conditioning, to assess neural activity associated with attentional processing of reward-related stimuli presented under capacity-limited conditions. We hypothesised that a CS+ distractor would induce an emotional attentional blink when presented at lag-2, impairing target detection, and aimed to explore the associated neural mechanisms. We predicted that the presence of CS+ images presented briefly within an RSVP stream would be associated with brain regions governing attention and salience such as the insula and amygdala, as well as areas of the value-driven attention network (caudate tail, IPS, LOC and early visual cortex). Correlations between neural activity and changes in subjective ratings from pre-conditioning to post-conditioning were also explored.

7.3 Methods and Materials

7.3.1 Participants

Twenty-four participants (12 males), aged 26.96 ± 4.66 (mean ± SD) completed the fMRI experiment. One female was later excluded from all analysis due to detection of a significant brain abnormality. Participants were required to be aged 18-40, fluent English speakers with normal or corrected to normal vision, and have a BMI between 18.5 and 30.0 (normal and overweight). Anyone with a history of neurological disease, eating disorders or diabetes were excluded from participation.

Participants were screened for any MRI contraindications by a radiologist at Liverpool Magnetic Resonance Imaging Centre (LiMRIC). The researcher then completed further medical screening with each participant, including a questionnaire to detect any food allergies or intolerances. Participants who declared any food allergy or intolerance were excluded from participation.

All participants gave full informed consent prior to the start of the experiment, in accordance with the Declaration of Helsinki. The experiment was given ethical approval by the University of Liverpool Research Ethics Committee.

7.3.2 Behavioural Tasks and Stimuli

7.3.2.1 Single-trial differential appetitive conditioning

Conditioning took place outside of the scanner in a separate, individual testing room. The CS+ and CS- were an edible object made from chocolate, and a plastic, inedible object, respectively. Objects were 3D novel geometric shapes, produced in either yellow or orange, designed to be neutral in valence, and have no prior conditioned association for the participants. The objects used were the same as those in Chapters 3-5, and the conditioning procedure was also consistent with that previously described. Colour-object assignment was counterbalanced across participants (see Table 7.1)

The CS+ was presented on a table in front of participants, on a small white plate. The CS- was presented in a small white plastic box. Presentation order was counterbalanced across participants. Participants were instructed to look at, handle and smell the first CS for two minutes (and to eat the CS+), whilst paying particular attention to the sensory characteristics of the objects. They then rated the sensory characteristics of the object on unipolar 100 mm VAS scales from 'not at all' to 'extremely'. Participants rated the appearance, smell and texture of both objects, as well as the taste of the CS+. The primary purpose of these ratings was to acquire taste ratings for the CS+. After a 90-second interval, they were then presented with the other CS, and the same procedure was followed.

Table 7.1 Counterbalancing of CS+ and CS- colour-object assignment

	Stimulus Type	
Group	CS+	CS-
1		
2		

7.3.2.2 Self-report measures of differential appetitive conditioning

Self-report measures of conditioned responses were taken once before, and once after conditioning. Participants were shown a photograph of both the CS+ and CS-, in a random order, and asked to complete three explicit measures of conditioning, using self-report 100 mm visual analogue scales (VAS), adapted from Papachristou et al. (2013). The questions were designed to capture conditioned cravings, CS-US expectancies, and changes in perceived pleasantness (see Table 7.2 for the wording of each question). The order of question type and stimulus type were randomised.

Table 7.2 Wording of questions and anchor points used on visual analogue scales presented during a self-report task where participants were required to rate the degree of pleasantness, cravings and expectancies elicited by a CS+ and CS-.

Measure	***Question***	***Anchors***
Cravings	*"When presented with this object, how strong is your craving for chocolate right now?"*	*"No craving at all" to "Extremely strong craving".*
US-Expectancy	*"When presented with this object, how strongly do you now expect to be invited to eat chocolate?"*	*"Certainly not" to "Certainly".*
Liking	*"How pleasant do you find this object?"*	*"Extremely unpleasant" to "Extremely pleasant"*

7.3.2.3 Questionnaires

Participants completed a battery of questionnaires to assess personality traits and attitudes related to reward sensitivity (BIS/BAS and Brief-SSS), food neophobia (FNS) and eating attitudes (DEBQ) consistent with previous chapters (see Chapter 2 [General Methods] for full descriptions).

7.3.3 fMRI Tasks and Stimuli

Participants completed a passive-viewing paradigm (both pre- and post-conditioning) and an EBA task (post-conditioning only) whilst in the scanner, over two separate

scanning sessions on a single test day. Both tasks were programmed and administered in PsychoPy2 v1.83.04 (Peirce et al., 2019).

7.3.3.1 Passive Viewing Task

Participants completed a passive viewing task once before conditioning, and once after conditioning. Each session was identical and consisted of images of the CS+ and CS- presented in an event-related design. Four images of the CS+ and four images of the CS- objects were created in Sketch Up (Trimble Inc, CA, USA). Images were 3D, visual representations of the CS+ and CS- objects on a plain grey background. Each image depicted the object from a different orientation to mimic normal viewing (i.e., front view, side view and 45° left and right). Each image was presented four times, with thirty-two image presentations in total (16 CS+, 16 CS-). Images were presented in a pseudo-random order with each CS type presented no more than twice consecutively.

Each trial began with a 2 s fixation cross, then an image of either the CS+ or CS- presented for 6s. Following each image, there was a staggered inter trial interval (ITI) of 7-11 s, when participants were presented with a blank screen, to allow hemodynamic responses to return to baseline before the next trial with the other CS began. The layout of a trial is depicted in Fig. 7.1 Participants were instructed simply to look at the images on the screen for the duration of the task, and to try not to fall asleep.

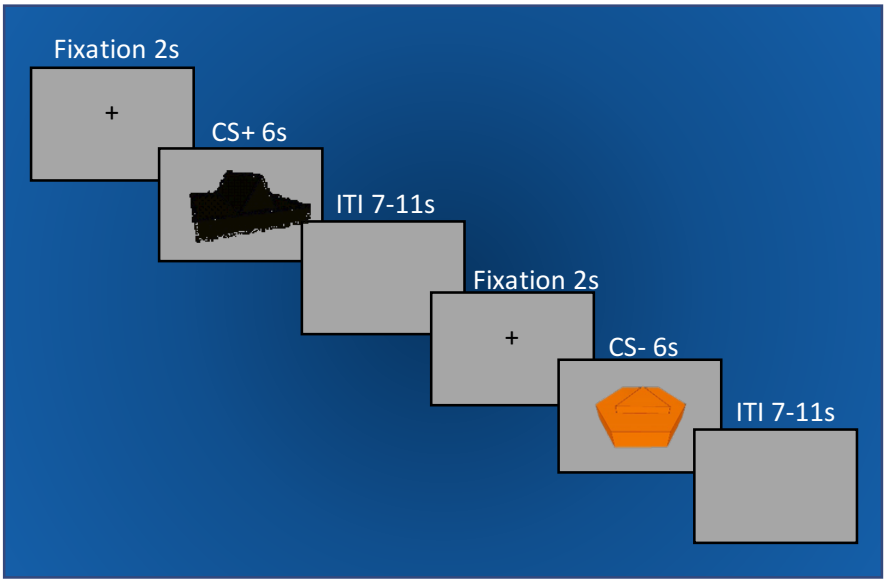


Fig. 7.1 Schematic representation of two consecutive trials on the passive viewing task.

7.3.3.2 Emotional Blink of Attention Task (EBA)

In each trial, a rapid serial visual presentation (RSVP) sequence was presented, consisting of 17 images presented for 100 ms each, with no inter-stimulus interval. Fifteen of these images were non-critical filler images (photographs of upright landscape scenes), selected from a battery of 256 images used in Chapters 3-6. The other two were of a critical distractor, which could occur at position 4, 6, 8 or 10, and a target image occurring 200 ms later (lag 2). Neutral distractors were 48 photographs randomly selected from the IAPS neutral database, which have been consistently shown to be neutral in valence (Lang, Bradley, & Cuthbert, 2001). Forty-eight photographs each of the CS+ and the CS- objects were taken against various everyday household backgrounds to provide similar complexity to the other task images. All images were resized to 320 × 240 pixels and matched for luminance.

At the start of a trial, a black fixation cross was shown for 1000 ms followed by a 1700 ms RSVP sequence. The screen then remained blank for 5000 ms, before responses were requested for two separate questions. Participants were first asked whether they had seen a rotated landscape image (Yes/No), and then which way the landscape image was turned (left/right) by using a ResponseGrip (Nordic Neuro Lab, Norway), an MRI-compatible subject response device. Participants held a response device in each hand and used their thumbs and index fingers to make responses when prompted on the screen (Right thumb = Yes; Left thumb = No; Right finger = Right; Left finger = Left). Maximal response time per question was 2000 ms. Trials continued in sequence regardless of whether a response was made. Even on trials where participants reported seeing no rotated target, they were still asked to make a response to the second question at random. Following the second question, there was a post-trial pause of between 8 and11 seconds duration when participants were asked simply to rest and await a fixation cross to signal the start of the next trial. The layout of a trial is depicted in Fig. 7.2. The first four trials inside the scanner were practice trials which were discarded from analysis, followed by a total of 48 trials (16 trials per condition). Overall trial duration was 19-23 seconds in total.

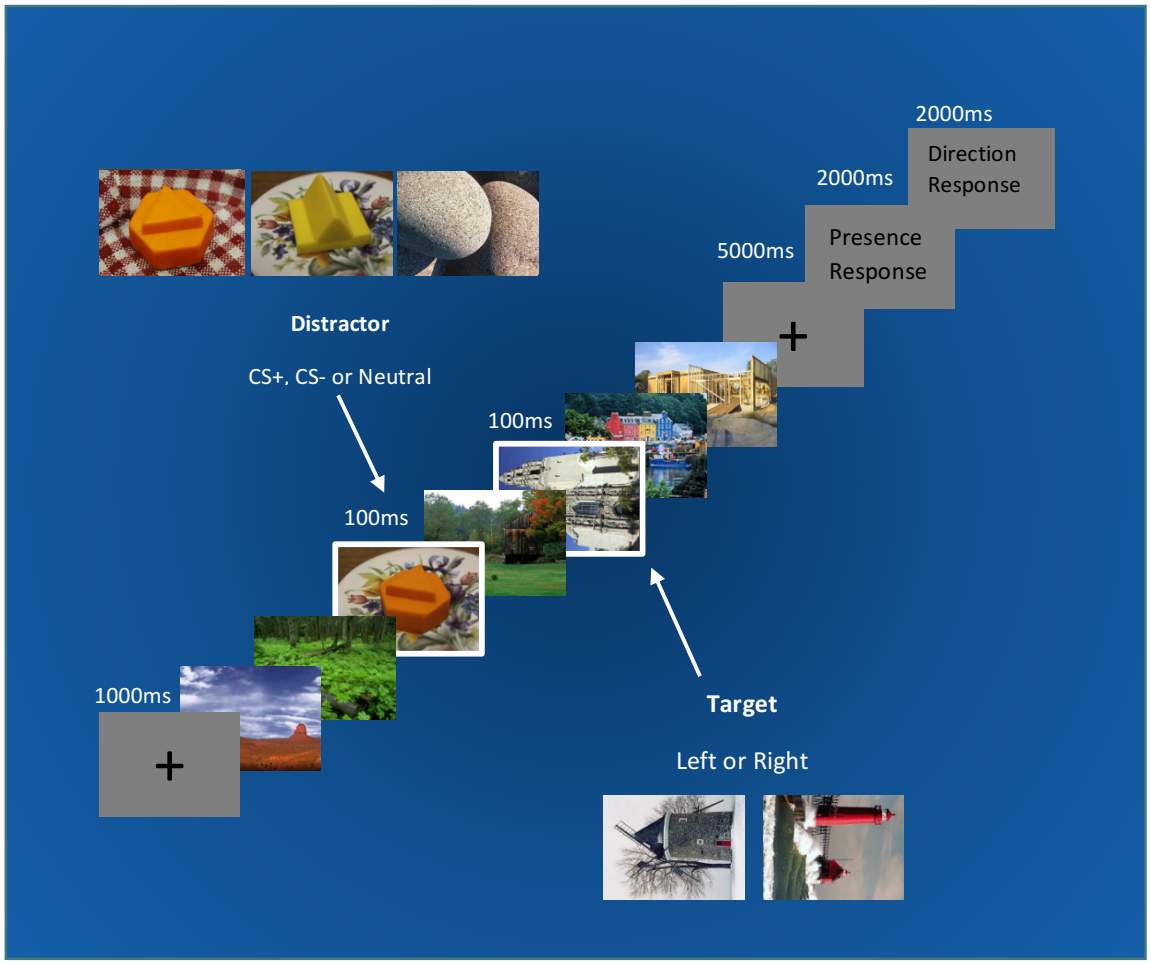


Fig. 7.2 Schematic representation of a single trial on the EBA task (Sequence of images has been shortened).

7.3.4 Procedure

An overview of the study procedure is illustrated in Figure 7.3. All testing took place between 10:30 and 16:30 in the Liverpool Magnetic Resonance Imaging Centre (LiMRIC) at the University of Liverpool. Participants were asked to refrain from eating or drinking (except water) for at least three hours immediately preceding their arrival at LiMRIC. This deprivation period was chosen to create a hunger state that most individuals experience as they approach their next meal, when, theoretically, motivation for food should be high, thus maximising reward value of the US.

After safety screening was carried about by a radiographer, participants completed four visual analogue scales (VAS) measuring hunger (as described in detail in Chapter 2). Pre-conditioning self-report measures of conditioned responses were then taken for the CS+ and CS-. Following removal of any external metal, such as jewellery, and items with metal fastenings, participants were brought in to the

fMRI room. Participants lay on the fMRI table outside of the machine, and were given ear plugs to minimise the noise from the scanner. Foam padding was placed around participants' heads to minimise movement during the experiment, and they were instructed to remain as still as possible throughout the experiment.

Stimuli were presented on a monitor located behind the centre of the scanner bore and viewed by a mirror mounted on the head coil. Adjustments to the positioning of the mirror were made for participants at the start of each session to ensure that the full monitor screen could be seen. Participants were given a safety buzzer to hold, and were instructed to squeeze the buzzer if at any point they felt uncomfortable to signal to the radiographer that the experiment needed to be stopped. During the first scanning session, participants first underwent diagnostic T1 (10 minutes) and T2 (2 minutes) weighted images, before undergoing functional scanning during a passive viewing task (10 minutes). Following this task, participants were brought out of the scanner to complete the conditioning phase of the experiment. After conditioning, participants repeated VAS ratings of hunger, and completed the three post-conditioning self-report measures of conditioned cravings, expectancies and pleasantness.

Prior to beginning the second session, participants were provided with task instructions for the EBA and seventeen practice trials were completed outside of the scanner to ensure they understood the task and had the opportunity to ask questions. Participants were given the opportunity to use the bathroom and have a drink of water, before returning to the fMRI room. Alignment with the positioning of the first scanning session was attempted as much as possible on the second scanning session. In the scanner, participants then completed a second localiser (90 seconds), the post-conditioning block of the passive viewing task – identical to the first (10 minutes), followed by the EBA task (20 minutes). Finally, participants were brought out of the scanner and completed a battery of questionnaires to provide descriptive statistics about the sample. Participants were asked to recall the colour and shape of the CS+ and CS- to check levels of contingency awareness, and were asked to describe the experimental aims. Height and weight were checked with scales and a stadiometer. Participants were given a thorough verbal and written debrief, and reimbursed £30.

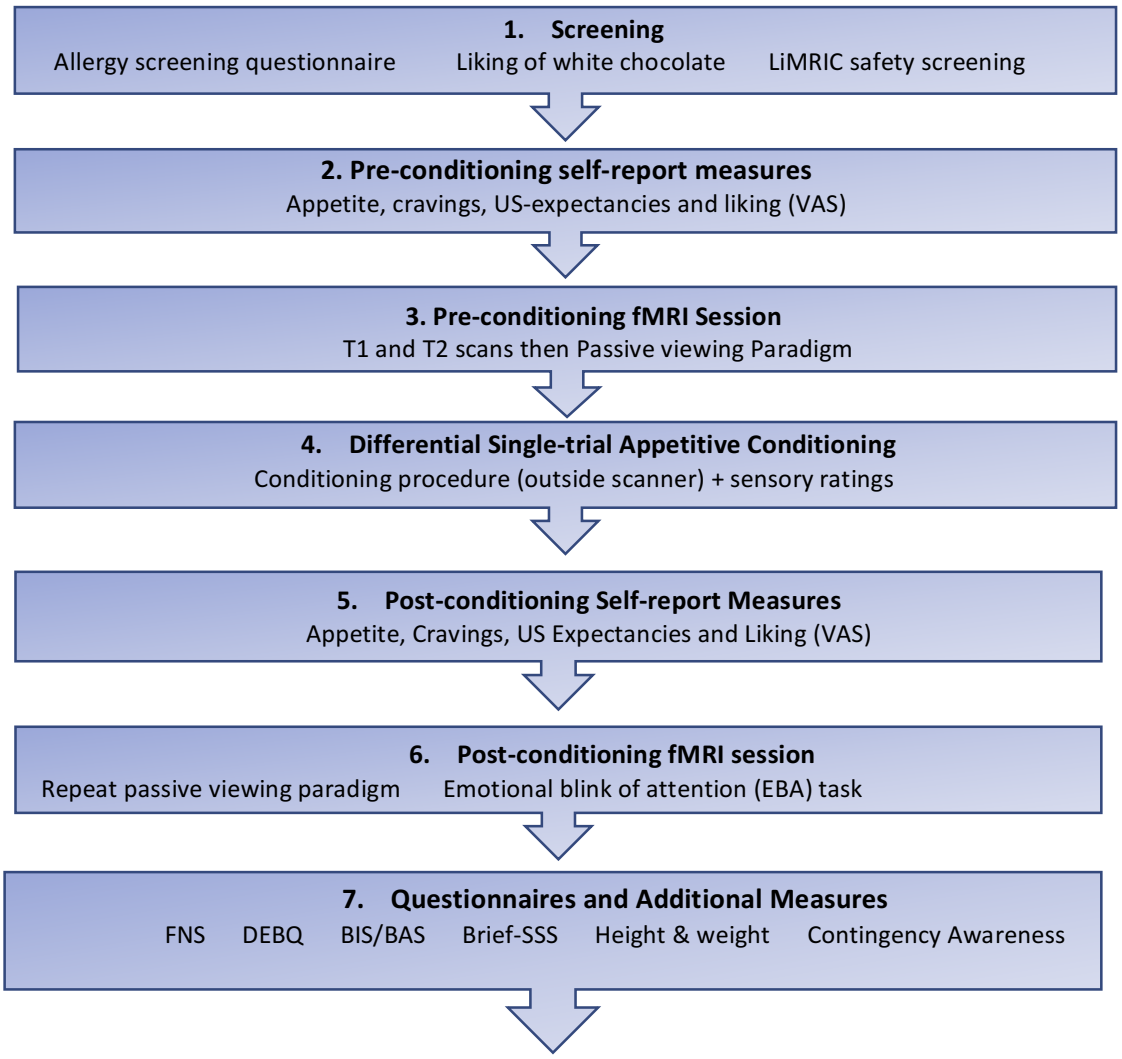


Figure 7.3. Flow chart depicting the study procedure

7.3.5 Image Acquisition

Magnetic resonance imaging scans were undertaken using a whole-body Tesla Siemens Trio 3T MRI imaging system (Siemens, Magnetom, Erlangen, Germany) and an 8-channel radiofrequency head coil.

As required by the LiMRIC safety protocol, a localiser scan (26 s) was acquired first, followed by a clinical T2 weighted anatomical scan. This scan was assessed by a clinician in order to identify incidental findings and other medical anomalies and was not collected for our investigation.

Following the clinical scan, a high-resolution, 3-dimensional, T1- weighted image was acquired using a Magnetization-Prepared Rapid Acquisition Gradient-Echo (MP-RAGE) sequence (time to repeat [TR] = 2000.0 ms; time to echo [TE] =

2.25 ms; flip angle = 8°; 192 sagittal slices; slice thickness = 1 mm; in-plane voxel size 1 mm × 1 mm × 1 mm; total acquisition time: 7:30 min). The MP-RAGE was used to position functional, blood oxygen level dependent (BOLD) sensitive data acquisition.

Functional MRI data from the first passive viewing task was performed using a T2 weighted sequence (48 interleaved axial slices, with no gap, TR = 3000.0 ms, TE = 30.0 ms, flip angle = 90 degrees, field of view = 192 mm, voxel size = 3.0 × 3.0 × 2.7 mm). Following the first functional scan, participants were removed from the scanner in order to complete the conditioning procedure. Upon returning to the scanner the next 2 fMRI scans had the same parameters as the pre-conditioning fMRI scan and were positioned with a localiser scan.

These scans were evaluated at the Walton Centre by a qualified radiologist for medical anomalies or incidental findings that would require follow-up investigation. These scans were not analysed as part of this experiment.

7.4 Data Acquisition, Reduction and Analysis

7.4.1 Behavioural data analysis

7.4.1.1 Participant characteristics

Al participants demonstrated awareness of the CS-US contingencies and none accurately guessed the aims of the experiment. Table 7.3 provides an overview of participant characteristics. In terms of personality variables, one particularly restrained eater was identified based on the restraint scale of the DEBQ. However, their exclusion did not alter the pattern of results so their data remain in the full analysis.

Levels of food neophobia were lower than those reported in a comparable sample of European university students (Mean ± SD = 29.39 ± 10.07; Fenko, Leufkens & van Hoof, 2015). Scores ranged from 12 - 49 and, using the cut-offs proposed by Previato and Behrens (2015), 61% of the sample can be classified as neutral (16.5-38.5), 30% as neophillic (<16.4) and 9% as neophobic (>38.6). Eating attitudes were also consistent with mean scores of a sample of non-clinical Dutch undergraduates on DEBQ subscales (van Strien, Herman & Anschutz, 2011), with

mean (± SD) scores for restraint, emotional eating and external eating being 2.47 ± 0.88, 2.48 ± 0.71 and 3.13 ± 0.51, respectively. Mean BAS-RR scores were comparable to those of moderate scorers in a previous non-clinical sample (16.83 ± 2.26; Alloy, et al. 2006). Total scores on the Brief-SSS were also similar to those of a UK University based convenience sample (3.01 ± 0.59; Eachus, 2004)

Participants arrived at the lab in a state of moderate hunger, and self-reported appetite remained unchanged from pre- (63.26 ± 14.39) to post-conditioning (67.24 ± 11.85), $t(22) = -1.44$, $p = .164$, suggesting that participants' appetite levels were consistently high across the experiment.

Average taste ratings for the CS+, taken during conditioning, were generally high (70.96 ± 20.80; min = 21, max = 100), suggesting consumption of the edible object was a pleasant experience and, therefore, the CS+ should have high reward value.

Table 7.3 Participant characteristics. Mean (SD).

Gender	
Female N (%)	11 (48%)
Age	26.78 (*4.68*)
BMI	24.68 (*3.24*)
DEBQ	
Restraint	2.33 (0.*87*)
Emotional	2.98 (0.*95*)
External	3.65 (0.*60*)
FNS	23.48 (10.14)
BIS/BAS	
BAS-RR	16.61 (*2.02*)
Brief-SSS	3.20 (0.*70*)

BMI = body mass index, DEBQ = Dutch Eating Behaviour Questionnaire; FNS = Food Neophobia Scale; BIS/BAS = Behavioral Inhibition System (BIS) and the Behavioral Activation System (BAS); Brief-SSS = Brief Sensation Seeking Scale. As the DEBQ has a distinct three-factor structure, a total score was not computed.

7.4.1.2 Self-report measures of conditioning

Cravings

A 2 × 2 repeated measures ANOVA revealed a significant main effect of time, $F(1, 22) = 51.60$, $p < .001$, $\eta_p^2 = .70$, a significant main effect of type, $F(1, 22) = 11.38$, $p = .003$, $\eta_p^2 = .34$, and an interaction between time (pre- or post-conditioning) and CS type (CS+ or CS-) on self-reported cravings (see Figure 7.4A), $F(1, 22) = 27.45$, $p < .001$, $\eta_p^2 = .555$.

Prior to conditioning, both the CS+ and CS- elicited similar levels of craving, ($p = .203$). After conditioning, the CS+ elicited significantly greater cravings than the CS- (33.61 ± 26.19) ($p < .001$), suggesting successful appetitive conditioning.

Self-reported cravings in response to the CS+ rose significantly from pre- to post-conditioning ($p < .001$). Although, there was a slight increase in cravings for the CS- from pre- to post-conditioning as well ($p < .035$).

US-expectancies

Similarly, there was a significant main effect of time, $F(1, 22) = 64.10$, $p < .001$, $\eta_p^2 = .74$, a main effect of CS type, $F(1, 22) = 9.73$, $p = .005$, $\eta_p^2 = .31$, and a significant interaction between time-point and CS type on expectancy for chocolate, $F(1, 22) = 22.36$, $p = .001$, $\eta_p^2 = .50$ (see Figure 7.4B).

Expectancies for chocolate were similar for the CS+ and CS- before conditioning ($p = .388$). After conditioning, the CS+ elicited significantly greater US-expectancies than the CS-, again suggestive of successful differential appetitive conditioning, ($p < .001$)

Expectancies for chocolate in response to the CS+ rose significantly from pre- to post-conditioning, ($p = .001$). However, for the CS-, expectancies for chocolate were consistently low both before and after conditioning, ($p = .094$)

Liking

A final 2 × 2 ANOVA revealed a significant main effect of time, $F(1, 22) = 5.97$, $p = .023$, $\eta_p^2 = .21$, and a main effect of CS type, $F(1, 22) = 11.11$, $p = .003$, $\eta_p^2 = .34$.

Again, the interaction between time-point and CS type on liking ratings was significant, $F(1, 22) = 5.85$, $p = .024$, $\eta_p^2 = .210$ (see Figure 7.4C).

The pattern of data was indicative of successful differential appetitive conditioning. Both the CS+ and CS- were equally liked prior to conditioning, ($p = .148$), but after conditioning the CS+ was liked significantly more than the CS- ($p < .001$).

Liking for the CS+ rose from pre-conditioning to post-conditioning, ($p < .001$), whereas ratings for the CS- did not change, ($p = .927$).

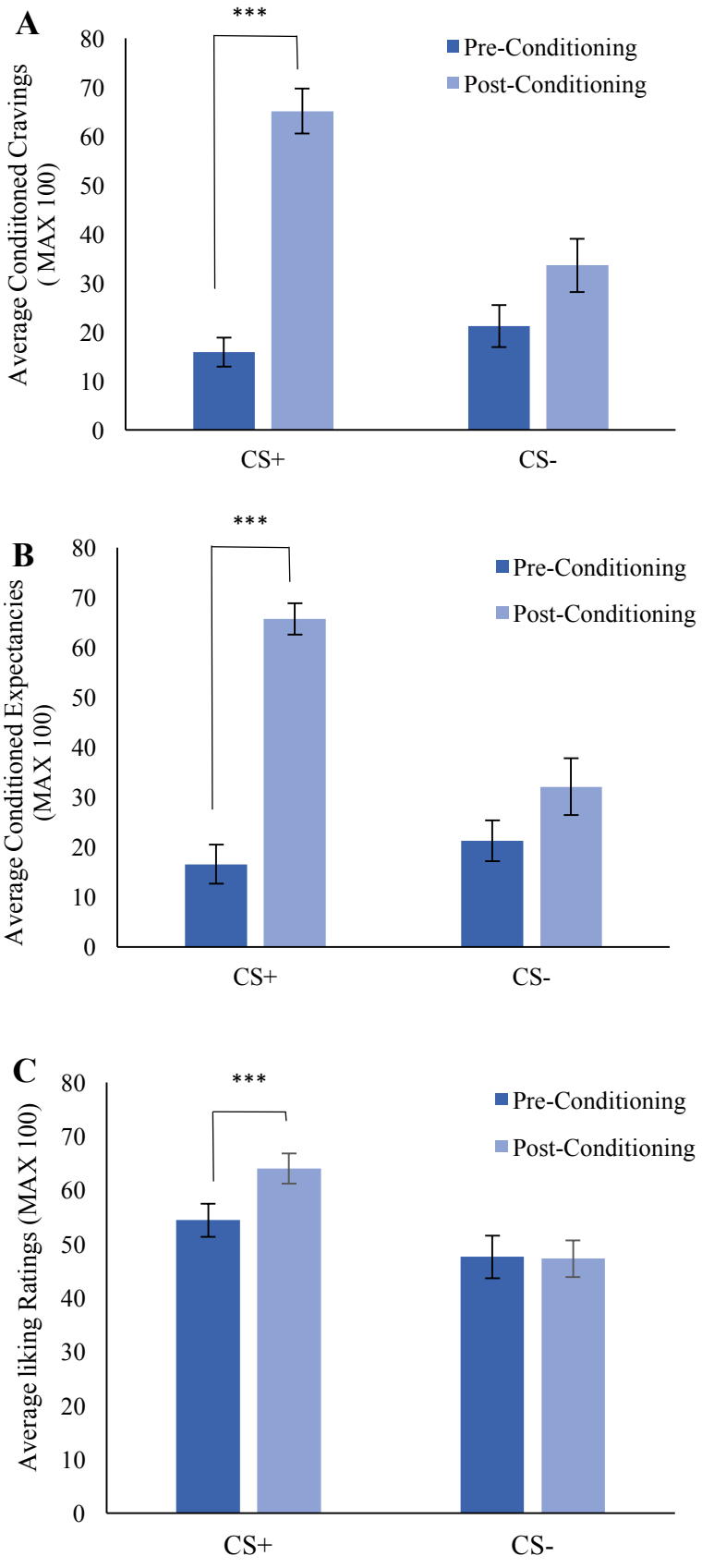


Figure 7.4 Average cravings (A), expectancies (B) and liking (C) ratings for the CS+ and CS- before and after conditioning. Bars represent mean ± SE. ***p<.001.

7.4.1.3 Emotional blink of attention task performance

Trials where participants identified both the presence and the direction of the target accurately were classed as correct, reducing the probability of correct responses by chance to just 25%. Missed or incorrect answers for either question rendered a trial incorrect even if the presence or direction were identified accurately. No reaction time cut off was applied, as each response window was limited to 2000 ms. The overall percentage of correct responses were calculated for each distractor category.

One way repeated-measures ANOVA revealed that levels of accuracy were similar for each distractor type on the EBA task, $F(2, 44) = 1.40$, $p = .257$, $\eta_p^2 = .060$, indicative of no differential attentional blink. (See Figure 7.5).

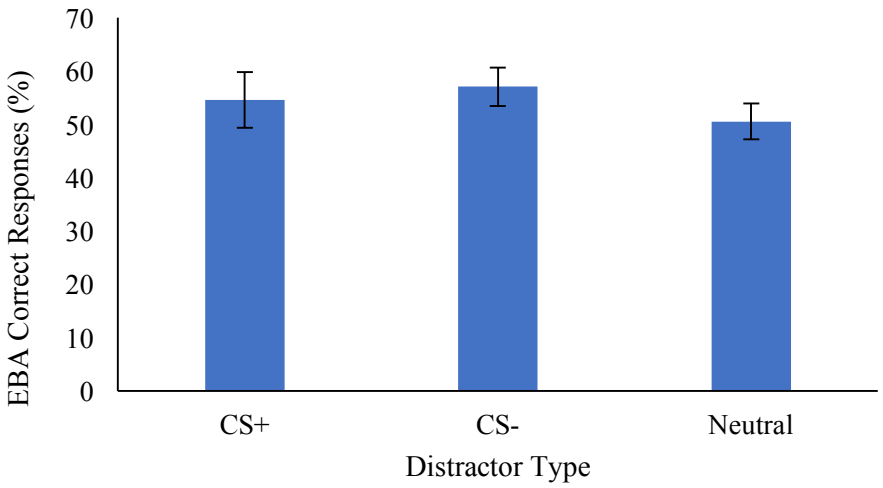


Figure 7.5 Percentage of correct responses on the EBA task for each distractor type.

7.4.2 fMRI data analysis

7.4.2.1 Pre-processing

The following pre-processing steps were applied to all functional volumes from the passive viewing task (190 volumes per task session) and the EBA task (390 volumes). DICOM (Digital Imaging and Communications in Medicine) data were converted to the NIfTI (Neuroimaging Informatics Technology Initiative) image format using MRI convert (Lewis Center for Neuroimaging, University of Oregon). Spatial pre-processing of functional data was then conducted using Statistical Parametric Mapping software package, SPM12 (UCL, UK:

www.fil.ion.ucl.ac.uk/spm) running on Matlab version R2018a (MathWorks Inc., Natick, MA).

All data reported are from scans with translational movement ≤ 3mm. Functional volumes underwent a standard statistical parametric mapping approach. Functional Images were slice-timing corrected, realigned, co-registered to the MP-RAGE structural image (which was then segmented). Following segmentation of the structural scan, functional scans could be normalized to EPI (Echo Planar Imaging) template image, and smoothed with an 8mm Gaussian Kernel with full width half maximum, to improve signal-to-noise ratio. A temporal high-pass filter was applied to the time series with a 128 second cut-off to remove low-frequency noise and slow drifts in signal.

7.4.2.2 Whole-brain analysis of the passive viewing task

Trials were modelled as events in a standard event-related design. For each functional block, 190 scans were entered into a first level design for each individual participant to define effects of condition. Six duration parameters were included as regressors. First level (i.e., at the individual level) contrasts were then computed to generate statistical parametric maps of the contrasts of interest (CS+ > CS-, Pre > Post, interaction). As the interaction between CS type and time-point allows for the most meaningful interpretation of the data, analysis is focused on this contrast and further explored, in line with our hypotheses.

At the second level, multiple comparisons were made across the contrast images of the interaction obtained for each participant, which were entered in to a univariate t-test. Given the exploratory nature of the research question, a minimum cluster size of 10 voxels (k = 10) was employed in the second level contrasts. A liberal uncorrected statistical threshold for the spatial extent test on the clusters was set at $p < 0.05$ at the cluster level over the whole brain, with a height threshold of $p < 0.001$. Peak voxels at significant clusters were selected as regions of interest (ROIs), and defined as 5 mm diameter spheres using MNI co-ordinates in the MarsBar 0.44 toolbox for SPM12 (http://marsbar.sourceforge.net/; Brett, Anton, Valabregue, & Poline, 2002). Contrast estimates for each condition were then extracted. These data were then tested further using a 2 × 2 repeated measures ANOVAs in SPSS, with a confirmatory threshold of $P < 0.05$. Significant interactions were explored with *post hoc* paired t-tests. Correlations were used to explore associations between neural

activity and behavioural responses. The coordinates derived from these analyses were converted from MNI coordinates to Talairach coordinates using the Yale BioImage Suite application (Lacadie et al., 2008), and labelled using Talairach Client (Lancaster et al., 2000) in order to associate findings with an anatomical location (Talairach & Tournoux, 1988). Labels were assigned according to the nearest grey matter position.

7.4.2.3 ROI analysis of the passive viewing task

As whole-brain analysis may be overly conservative, particularly whilst investigating such rapid learning, region of interest analysis was also performed at regions associated with reward-processing and attention. Reward-related amygdala, NAcc, OFC and ACC ROIs were defined using a meta-analysis with the search term 'reward' in Neurosynth and selecting MNI co-ordinates at maximal intensity. Areas related to value-driven attention were defined based on coordinates presented in the existing literature: caudate tail (Du et al., 2013), lateral occipital cortex (Padmala, Sirbu & Pessoa, 2017), IPS (Coutlee et al., 2016) and extrastriate cortex (Kätsyri, Hari, Ravaja & Nummenmaa, 2013) (See Table G1, Appendix G for MNI coordinates). Using the MNI coordinates, spherical ROIs were created in MarsBar and ROI analysis was conducted using the interaction contrast.

7.4.2.4 Whole-brain analysis of the EBA task

After pre-processing, 390 scans were entered into the first level design for each participant where stimulus onsets and durations of each trial type were entered. This resulted in six movement parameters being included as regressors in the first level model. Paired t-tests were conducted at the first level for three main contrasts of interest (CS+ > CS-, CS+ > Neutral, CS- > Neutral) across the whole brain.

Due to the relatively small number of EBA trials, and the fact that performance was comparable for each distractor type, responses were not analysed based on accuracy, which would have reduced the power considerably. In the second level, one sample t-tests were conducted at group level for each of the contrasts determined in level one. We used the same statistical threshold as that in the passive viewing task. Two ROIs (10 mm spheres centred on the peak activation) were identified from the CS+ > CS- contrast and two from the CS+ > Neutral contrast.

Contrast estimates for each ROI were correlated with behavioural measures and individual differences.

7.4.2.5 ROI analysis of the EBA task

Again, ROI analysis was conducted based on pre-defined brain regions, following the same process outlined above (7.4.2.3).

7.5 fMRI Results

7.5.1 Passive viewing task results

7.5.1.1 Whole brain analysis of the passive viewing task

Whole brain analysis at an uncorrected threshold of $P<.001$, with a height threshold of 3.50 and an extent threshold of >10 voxels revealed a single significant cluster of 18 voxels in the frontal lobe with a peak voxel in the superior frontal gyrus, (see table 7.4 and figure 7.6).

Table 7.4 Whole brain analysis: t-contrast of the interaction between time (pre- and post-conditioning) and CS type (CS+ and CS-) in a passive viewing task.

Anatomical Location	MNI x, y, z	Cluster size (k)	T-value	P-value	Z
R.SFG	32 64 2	18	4.35	0.039	3.65

R.SFG = Right superior frontal gyrus. The statistical threshold was set at $p < 0.05$ at the cluster level, with a height threshold of $p < 0.001$. K = cluster size (voxels). MNI = Montreal Neurological Institute. MNI coordinates refer to the peak activated voxels.

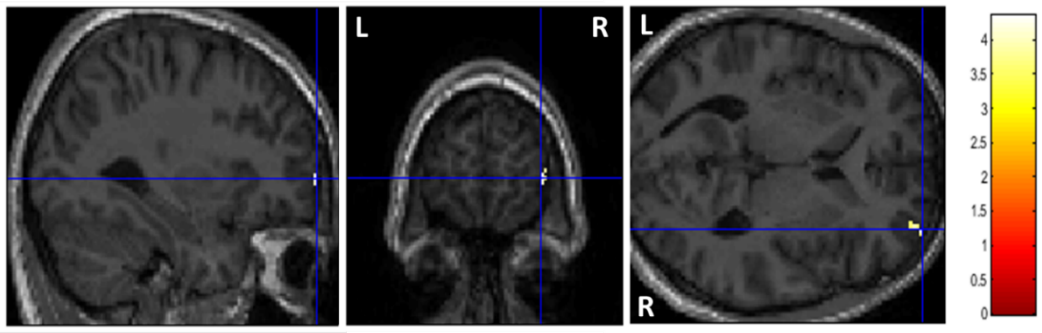


Figure 7.6 A contrast showing the interaction effect between stimulus type (CS+/CS-) and session (pre- and post-conditioning) in the superior frontal gyrus (coordinates and values shown in table 7.1). 'L' and 'R' represent left and right hemisphere respectively.

Using MarsBar, a 5.0 mm sphere at these MNI coordinates was selected as a functional ROI. Following this, mean contrast estimates were obtained for each of the four conditions (Pre-conditioning_CS+, Pre-conditioning_CS-, Post-conditioning_CS+ and Post-conditioning_CS-). These mean contrast estimates were then entered in to a 2 × 2 repeated measures ANOVA using SPSS. The criterion for significance was set to $p < .05$. This revealed a significant interaction between CS type and time point, $F(1, 22) = 7.73$, $p = .011$, $\eta_p^2 = .260$. Paired t-tests revealed no change in contrast estimates from pre- to post-conditioning for the CS+, $t(22) = .641$, $p = .528$. However, for the CS-, activity in the R.SFG significantly increased from pre- to post-conditioning, $t(22) = 2.34$, $p = .029$, (see figure 7.7)

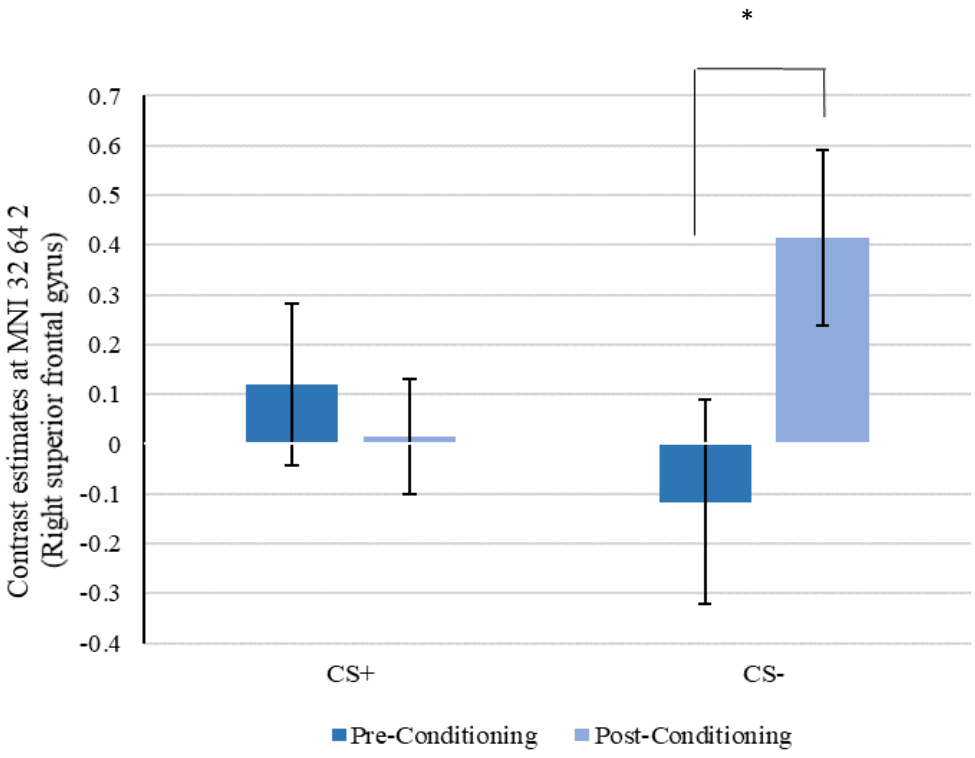


Figure 7.7 Mean (± SE) BOLD magnitude for each condition (CS+_Pre, CS+_Post, CS-_Pre, CS-_Post) within peak MNI coordinates (*x*, *y*, and *z*) of the functional cluster. * $P < 0.05$. MNI = Montreal Neurological Institute.

7.5.1.2 ROI Analysis of the passive viewing task

No significant clusters were identified for the interaction contrast at *a priori* regions of interest.

7.5.1.3 Associations between BOLD signal, behavioural measures and individual differences

Difference scores were calculated for the change in BOLD activation at the R.SFG in response to visual presentation of the CS- before and after conditioning, as well as for each of the self-report measures (expectancy, cravings and liking) of the CS- stimulus. After applying appropriate statistical corrections, no significant associations between change in neural activity and self-report measures of conditioning (See Table G2, Appendix G for correlation matrix).

7.5.2 EBA task results

7.5.2.1 Whole brain analysis of the EBA task

We initially explored overall activation patterns in the EBA task by comparing the effect of distractor type using three T-contrasts: CS+ > CS-, CS+ > Neutral, CS- > Neutral. The t-contrast of CS+ relative to CS- distractors revealed activation in the right insula and left cuneus (see Figure 7.8 and Table 7.5). The t-contrast of CS+ vs. Neutral revealed activation in the left superior temporal gyrus and left middle temporal gyrus (see Figure 7.9 and Table 7.5). In contrast, the CS- vs. Neutral distractor comparison showed no significant activation.

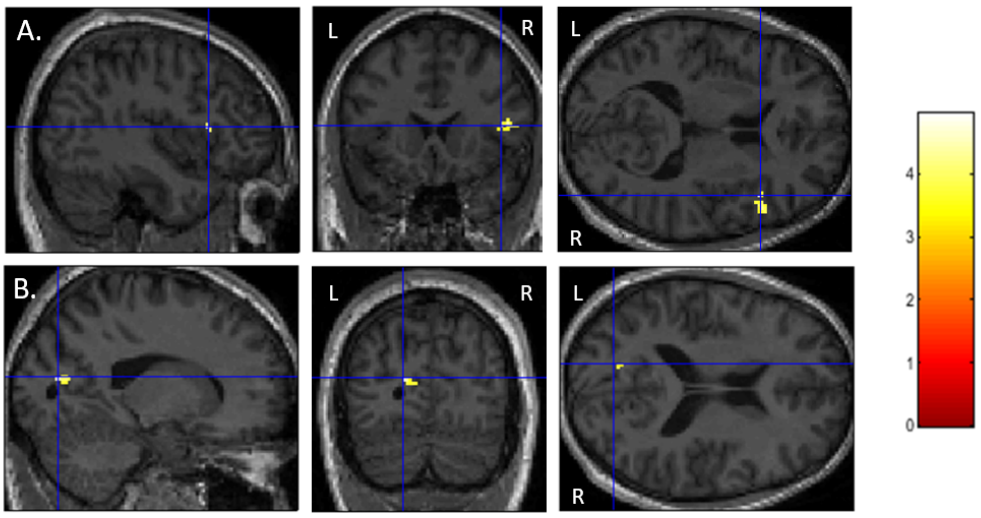


Figure 7.8 A t-contrast (CS+ > CS-) revealing clusters in (A) right insula and (B) left cuneus which were defined as ROIs (co-ordinates and values are shown in Table 7.5). 'L' and 'R' represent left and right hemisphere, respectively.

Table 7.5 Whole brain analysis of an EBA task: t-contrasts of distractor type

Contrast	Anatomical Location	MNI x, y, z	Cluster size (k)	T-value	P-value	Z
CS+ > CS-	R. Insula	42 18 10	36	4.95	.019	4.01
	L. Cuneus	-16 -78 14	35	4.53	.020	3.77
CS+ > N	L. STG	-46 0 -20	28	5.22	.047	4.17
	L. MTG	-48 -74 22	36	4.36	.027	3.66

MNI = Montreal Neurological Institute. K = cluster size (voxels). R = right, L = left. STG = superior temporal gyrus. MTG = middle temporal gyrus. The statistical threshold was set at $p < 0.05$, at the cluster level, with a height threshold of $p < 0.001$. MNI coordinates refer to the peak activated voxels.

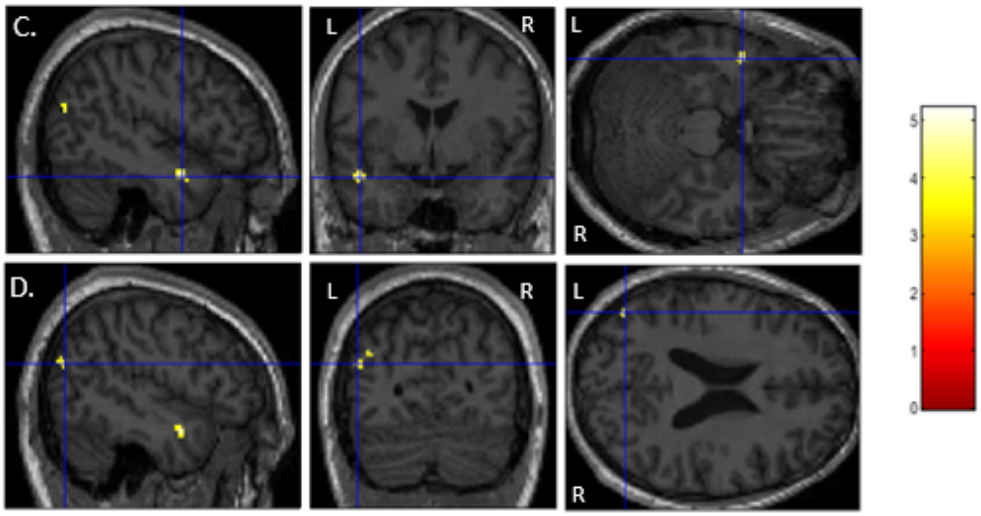


Figure 7.9 A t-contrast (CS+ > Neutral) revealing clusters in (C) left superior temporal gyrus and (D) left middle temporal gyrus which were defined as ROIs (co-ordinates and values are shown in Table 7.). 'L' and 'R' represent left and right hemisphere, respectively.

7.5.2.2 ROI analysis of the EBA task

ROI analysis was conducted to test our hypothesis in seven theoretically driven regions known to be involved in reward and attention. No significant clusters were identified for the t-contrasts at any of the *a priori* regions of interest.

7.5.2.3 Associations between BOLD signal, behavioural measures and individual differences

After applying corrections for multiple comparisons no correlations reached statistical significance (See Table G3, Appendix G for correlation matrix).

7.6 Discussion

The aim of this study was to investigate the neural correlates of single-trial appetitive conditioning in healthy subjects using a novel, naturalistic conditioning paradigm, designed to mimic a realistic encounter with a new food. After participants ate a CS+ object (made from chocolate), subjective responses revealed clear evidence for differential appetitive conditioning in a single trial. Participants were more likely to expect to receive chocolate upon subsequent presentations of the CS+, indicating an awareness of the CS_US contingency. The CS+ was also perceived as more pleasant and elicited cravings for chocolate post-conditioning. Similar changes were not observed for a CS- (made from plastic).

Contrary to our predictions, exploratory whole brain analysis showed that this conditioning paradigm did not modulate neural activity in response to a CS+ during passive viewing. Unexpectedly, however, our single-trial conditioning paradigm was associated with a small yet significant increase in neural activity in the right superior frontal gyrus (R.SFG) in response to the sight of the CS-. BOLD activation in this area was, however, unrelated to subjective measures of CS-US expectancies, cravings and pleasantness.

The ability to select appropriate behavioural responses and to suppress action when necessary is an essential skill which can prevent distraction and unnecessary action. Research has begun to uncover distinct neural networks involved in such response inhibition, to which the frontal lobes make a major contribution (Hampshire et al., 2010; Sharp et al., 2010; Walther et al., 2011). In particular, a negative association between cortical thickness of the R.SFG and scores on the Barratt-Impulsiveness-Scale suggest a role of the R.SFG in mediating impulsivity and behavioural inhibition (Schilling et al., 2012). Dambacher et al. (2014a) confirmed that the R.SFG is part of a distinct neural network involved in action restraint (Dambacher et al., 2014a). Using transcranial magnetic brain stimulation, continuous theta-burst stimulation disruption of activity in the R.SFG impaired performance on a go/no-go task, which relies on participants inhibiting a response before it is made

(Dambacher et al., 2014b). The ability to cancel an action already underway was unaffected. Furthermore, in a stop signal task Hu, Ide, Zhang & Li (2016) report that increased activity in the R.SFG was associated with more efficient response inhibition, as well as less motor urgency. Consequently, the R.SFG appears to play a significant role in response inhibition and inhibitory control.

However, the present study is the first to suggest a role of the R.SFG in differential appetitive conditioning. The increase in activity at the R.SFG during post-conditioning presentations of CS- images, but not CS+, might suggest that after differential learning, the brain can discriminate between a motivationally relevant CS+, paired with food, and an irrelevant CS- which lacks any reward associations and consequently inhibits responding to this unimportant stimulus. As a result, further cognitive processing may be inhibited and approach motivated behaviour prevented. Once a neutral stimulus has been found to lack reward associations it would be disadvantageous to continue exploring it further, so this may represent an adaptive mechanism ensuring efficient motivated behaviour. This novel finding is of particular importance given that modulation of R.SFG activity occurred after just a single trial with naturalistic stimuli.

It is worth noting that neural activity was only recorded before and after conditioning took place; the actual conditioning procedure took place outside of the scanner. As such, the activity observed following conditioning relates to the outcome of learning, and may not specifically inform us about the neural processes involved in the acquisition of single-trial appetitive conditioning. Current evidence suggests that the neural regions involved in acquisition of learning differ from those involved in outcome learning measured at a later stage (Karuza, Emberson & Aslin, 2014). Whilst it was not possible, due to scanner restraints, to measure neural activity during conditioning, it may be worth exploring other paradigms that would allow for this in future research. Similarly, subjective measures of conditioning were also completed outside of the scanner. Recording activity while evaluative judgements are being made might provide additional, critical information regarding the neural basis of appetitive conditioning.

We found no behavioural evidence of reward-driven attentional capture based on EBA responses. Participants detected a target in an RSVP stream at lag-2 with a similar degree of accuracy regardless of the preceding distractor type. Based on work by Smith et al. (2006), demonstrating the utility of the EBA for reflecting moment to

moment changes in stimulus value, the present data would appear to indicate that the CS+ failed to induce an 'attentional blink' to any greater extent than stimuli lacking any reward associations. Nonetheless, the brief presentation of a CS+ distractor in the EBA elicited greater activation in the right insula and the cuneus area of the left occipital lobe, than did a CS- distractor. Furthermore, compared to IAPS neutral distractors, CS+ distractors also elicited greater activation in the superior temporal gyrus and the middle temporal gyrus. However, it is again important to note that these effects were only evident at an uncorrected threshold and thus, results must be interpreted with caution.

Consistent with previous work, the involvement of each of these regions suggest that attention to, and saliency of, the CS+ distractors may have been greater than for CS- and neutral distractors (Rapuano et al., 2016; Gearhardt et al., 2013). More specifically, the STG has been implicated in salience detection and shows a heightened sensitivity to novelty (Downar, Crawley, Mikulis & Davis, 2002), whilst the MTG is thought to be involved in object processing and recognition (Holdstock, 2005) as well as semantic processing of pictures and words (Taikh, Hargreaves, Yap & Pexman, 2014). Thus, it may be that the enhanced activity seen in these regions in comparison to neutral distractors is reflective of recognition of a motivationally salient object and the subsequent processing of its associated meaning.

The involvement of the right insula is consistent with previous research conducted by Schwabe et al. (2011) in which anterior parts of the ventral attention network (VN), including the anterior insula, lateral OFC and dorsal anterior cingulate (dACC) were activated more strongly across trials in which an emotional T1 interfered with detection of a second target. The fact that insula activity in the present EBA task was strongest on trials in which a CS+ distractor was present may suggest that the VN was involved in detecting and orienting attention in a stimulus-driven fashion to the most behaviourally relevant stimulus.

The insula is also considered to be part of a 'salience network', responsible for the detection of salient stimuli and initiating appropriate attentional control signals (Menon & Uddin, 2010). There is thought to be a high degree of overlap between both anatomy and function of the SN and VN, with some arguing they are part of the same system (Kucyi, Hodaie & Davis, 2012) and share a role in responding to salient stimuli which have motivational relevance to the individual (Farrant & Uddin, 2015).

In a typical 'oddball' paradigm, the insula is consistently activated when novel or deviant stimuli interrupt a continuous stream (e.g., Chen, Lee & Cheng, 2014), suggesting that the insula processes salience at a pre-attentive level. After salient stimuli are detected, the SN then engages higher cognitive processes such as memory, attention and action selection processes (Harsay, Spaan, Wijnen & Ridderinkhof, 2012).

Determining whether a stimulus is salient is a subjective process determined by a variety of factors. Given the insula's complex role in sensory, affective, motivational, attentional and decision-making processes, Uddin et al. (2017) propose that the function of the insula can be best summarised as filtering competing stimuli to identify the most homeostatically relevant item.

The right insula has also been heavily implicated in gustatory processing and taste discrimination (Barry, Gatenby, Zeiger & Gore, 2001) as well as generating somatosensory representations related to mouthfeel and texture of food (Rolls, 2016). We might speculate that the sight of a CS+ can elicit taste representations of the associated US. Further research is necessary to better understand these neural mechanisms, and their contribution to eating behaviour.

Finally, the cuneus is a wedge-shaped area of the occipital lobe, which plays a crucial role in primary visual processing (Cohen, 2011). Research has also shown that the cuneus is sensitive to reward (Thomas, Vanni-Mercier & Dreber, 2013); reward magnitude modulated activity in this region (Delgado, Locke, Stenger & Fiez, 2003), with greater activity in response to reward anticipation (Vaidya et al., 2013).

Several features of the experimental design are worth consideration. Despite completing a separate practice of the EBA task outside of the scanner, participants completed only four practice trials inside the scanner to minimise task duration. Due to the inherently high noise levels, participants were unable to communicate with the experimenter during scanning. Consequently, once the task began it was not possible for questions to be asked without halting the experiment. Furthermore, due to scanner constraints, having learned the EBA procedure using a standard laptop keyboard during the practice, participants were required to switch to using an fMRI compatible handheld ResponseGrip in the scanner. Different input devices place varied demands on attentional resources and can have a significant impact on performance (McLaughlin, Rogers & Fisk, 2009). It is unknown how different input

devices may have impacted on task difficulty and performance in the present experiment, but perhaps indicates why we failed to show behavioural differences in task performance on the EBA task.

Furthermore, whilst scanning procedures are generally well tolerated, excessive noise and lengthy procedures can add to perceived discomfort (Szameitat, Shen & Sterr, 2009), perhaps interfering with concentration and performance. With over seventy minutes in total inside the scanner during testing, this was a rather lengthy protocol and the requirement to stay very still may have accentuated any discomfort and boredom, further impairing performance. This combination of factors may have resulted in a floor-effect due to overall task difficulty. Future research may benefit from the use of personalised procedures which calibrate task difficulty based on individual performance (e.g., Lim, Padmala & Pessoa, 2009)

Another potential limitation of the present experiment was the relatively small sample size (final sample: n=23). This may have been particularly problematic for the EBA task; due to practical constraints and the lengthy protocol, the number of EBA trials presented was reduced from standard laboratory experiments in order to minimise fatigue and discomfort during scanning. Consequently, the power to detect effects may have been lacking, resulting in small effects which did not survive corrections, as well as a failure to replicate prior findings related to attention and reward related regions of interest. An investigation by David et al. (2013) highlighted how power is a common problem in fMRI research with the majority of studies including only small sample sizes (N<40), often due to cost and resource constraints, and thus are underpowered. Reporting biases, particularly in small studies, may enhance small or incidental findings, and null results may go unpublished. Hence, commonly accepted findings regarding reward-related processed may be overstated, and it is likely that a number of similar studies have gone unreported due to publication bias.

In summary, replication of the modulation of self-reported expectancies, cravings and liking further highlights the utility of this novel, naturalistic procedure for studying single-trial conditioning in humans and could have important implications for understanding human food-cue reactivity. This study also provides a first insight into the neural mechanisms underlying single-trial appetitive conditioning. Contrary to predictions, no evidence of changes to implicit attentional processing emerged at a behavioural level, yet the fMRI data support the

involvement of key areas related to attention and saliency detection when CS+ distractors are present, relative to CS- or neutral distractors. Additionally, we make a novel contribution to the field by identifying a role for the right superior frontal gyrus in differential appetitive conditioning, specific to learning about a CS- with no reward associations. The pattern of activity we observed reveals a potential role of this structure in inhibiting action. This study provides a first look at the neural correlates of naturalistic single-trial appetitive conditioning in humans and highlights important insights which warrant further inquiry.

Chapter 8

General Discussion.

8.1 Overview of aims

The present thesis explores how learning shapes motivational and attentional processing of food-related cues. Food cues can acquire incentive motivational properties via appetitive conditioning, potentially leading to cue-elicited craving and overeating. Thus, learning processes are considered central to the development and maintenance of obesity and certain eating disorders. Consequently, it is essential from a theoretical perspective that we broaden our understanding of these processes, and their contribution to human eating behaviour. In addition, clinically, this understanding may have important applications for informing treatments and interventions with the potential to prevent or diminish aberrant cue-reactivity.

Chapter 1 summarised the present literature regarding the contribution of learning and motivation to the control of appetitive behaviour. These systems were then related to a relatively new area of research regarding reward-driven attentional capture. That chapter highlighted the relative difficulty of studying appetitive conditioning, with the selection of a suitable US, and an effective paradigm posing significant challenges. Nonetheless, with a sufficiently powerful paradigm, involving consumption of a novel, palatable food object, Blechert et al. (2016) suggested that reward learning was apparent after just a single trial, again highlighting the potential for these processes to have a significant impact on eating behaviour. As that study was the only one of its kind, it was clear that further exploration of this new paradigm was warranted.

Consequently, a primary aim of this thesis was to explore the utility of Blechert et al.'s single-trial naturalistic conditioning procedure for producing conditioned appetitive responses. The acquisition of conditioned responses was measured at both an explicit level, via self-report measures of craving, liking and expectancies for novel foods, and at an implicit level via changes in attentional processing of conditioned cues. This aim was explored throughout Chapters 3 to 7 with a single-trial conditioning procedure, as well as across multiple trials in Chapter

5 (Experiment 4). Generalisation of conditioned responses to similar but non-conditioned cues was explored in Chapter 6 (Experiment 5). Through exploration of the primary aim, a further objective arose, explored in Chapter 4: to unpick the role of reward learning, novelty and general salience in determining automatic attentional capture by newly encountered objects (Experiment 3). A final aim, explored in Chapter 7, was to shed light on the neural mechanisms underlying single trial appetitive conditioning, using fMRI (Experiment 6).

8.2 Can a naturalistic appetitive conditioning paradigm result in successful acquisition of conditioned responses in a just a single trial?

Traditional models of Pavlovian conditioning posit that learning is a gradual process occurring in increments across multiple trials (Rescorla-Wagner, 1972). Few studies have sought to explore how fast learning might take place. This thesis made a novel contribution to the field by investigating the phenomenon of single-trial appetitive conditioning through replication and extension of an adapted naturalistic conditioning paradigm, first described by Blechert et al. (2016). This conditioning procedure was designed to resemble a true real-world encounter with a new food-source. The sight of an edible object (CS) became associated with a sweet taste (US) as participants ate a novel edible object to form a CS+. As a test of differential appetitive conditioning, participants also saw and handled a plastic object, signalling no reward (CS-). Across this thesis, subjective (Chapters 3-7), attentional (Chapters 3-7) and neural (fMRI; Chapter 7) measures provided unique insights in to the explicit and implicit processes underlying single-trial conditioning.

8.2.1 Subjective measures of naturalistic single-trial conditioning

Chapters 4-7 provide consistent evidence for the acquisition of subjective conditioned responses in a single trial. Subjective cravings, US-expectancies and liking ratings increased for a food-paired object (CS+), but not a visually similar plastic-paired object (CS-) signalling no reward, after just a single trial, building on prior work in this field (Papachristou et al., 2013; Van Gucht et al., 2010). These findings support the validity of this naturalistic conditioning paradigm and further

support the assertion that conditioning plays a key role in food-cue reactivity (e.g., Jansen, 1998).

The possibility for such rapid learning is likely to be highly advantageous; in our evolutionary past, reliable food sources were scarce, and the costs of overlooking even one opportunity for nutriment could have been high. In such harsh conditions, the sight of food would signal survival, and associations between its appearance, and its nutrient value would likely prepare the individual for consumption – eliciting desire, cravings and approach-motivated behaviours. Patience and restraint would be risky, as one would never be sure of future food availability. Foods high in energy density would need to be quickly remembered for efficient detection and selection in the future, supporting the likelihood of rapid reward learning in just a single trial.

It was posited that the relative lack of evidence in the current literature for single-trial learning with appetitive compared to aversive stimuli was due to an absence of suitable appetitive stimuli of sufficient reward value. This argument is consistent with our repeated finding that a novel geometric object made from chocolate became a desirable, craved food after consumption in Chapters 4-7. In Chapter 3, when marzipan served as a US, as opposed to chocolate, evidence for conditioning was absent. Exploratory analysis presented in Experiment 2 suggested this failure could be attributed to the relatively low enjoyment of the US for some participants, suggesting low reward value. However, when a different US was chosen (chocolate), this was generally well liked, and differential appetitive conditioning was consistently present (Chapters 4-7) supporting the notion that the palatability of a food-US can influence the magnitude of a conditioned response (Armel et al., 2009).

Contrary to the common assumption that Pavlovian conditioning is a gradual process requiring repeated pairings to strengthen associations over multiple trials (Rescorla-Wagner, 1972), the results presented in Chapter 5 suggest that the greatest learning occurs in the first trial. Consistent with the abrupt 'all or none' pattern of learning proposed by Gallistel, Fairhurst & Balsam (2004), craving, US-expectancies and liking increased considerably after the first learning trial, yet additional conditioning trials failed to enhance conditioned responses beyond this initial experience. Thus, we demonstrate a powerful naturalistic paradigm for studying appetitive conditioning processes without the need for lengthy and repetitive

procedures, and provide further support for the existence and potency of single-trial appetitive conditioning.

8.2.2 Generalisation of subjective conditioned responses

Another important finding was that subjective conditioned responses can spread to stimuli similar to the CS+ via stimulus generalisation, despite never being directly paired with a US (Cuvo, 2003). The strength of the response is strongest for stimuli highly similar to the CS+ and diminishes as similarity to the CS+ decreases. For example, Lissek et al. (2008) presented 10 rings varying in size, with the CS+ and CS- as extremes, paired with an aversive image. The researchers demonstrated that conditioned fear fell along a downward similarity gradient, with the greatest startle response and negative subjective ratings linked to the CS+ and a continuous decrease in these as stimulus similarity to the CS+ decreased.

Chapter 6 presents an adaptation of this paradigm for an appetitive CS+. A novel geometric CS+ and CS- served as extremes, with six intermediary stimuli serving as generalisation stimuli (GSs), thus creating a similarity continuum from the CS+ to CS-.

Self-report ratings highlighted that acquisition of conditioned responses to the CS+ showed strong generalisation to stimuli most similar to the CS+, but decreased in a linear fashion as the GS became less similar to the CS+. This paradigm provides a novel, effective and adaptable tool for studying stimulus generalisation with an appetitive US which has, so far, received little attention.

Generalisation is thought to be an adaptive process. It is generally the case that similar events share some causal relationship, so from an evolutionary perspective it is highly beneficial for individuals to be able to use this knowledge to make predictions about novel situations based on familiar ones (Ghirlanda & Enquist, 2006).

In the harsh landscapes in which our ancestors evolved, food was scarce and opportunities to acquire nutriment could not afford to be missed. Thus, the ability to generalise learning about a food source, such as the colour of a nutritious fruit, may have proven essential for survival, ensuring potential new food sources were approached and explored.

However, if generalisation were to occur to a disproportionate degree – to only closely related stimuli, this process could pose a significant problem. Just as

excessive fear generalisation has been implicated in anxiety and panic disorders (Struyf, Zaman, Hermans & Vervliet, 2017), it seems plausible that excessive generalisation with appetitive stimuli could drive reward-related dysfunction such as excessive overeating, drug addiction and pathological gambling, particularly as the present thesis demonstrates that this phenomenon can occur with a single trial.

8.3 Reward-driven Attentional Capture: Does attentional capture reflect incentive salience of a reward paired cue?

A core assumption of this thesis was that food-paired cues would acquire incentive salience and thus attract attention based on their associations with reward. This notion draws on research from the field of vision science, which indicates that rewards modulate attentional processing via the mechanism of value-driven attentional capture (Anderson et al., 2012). Thus, it was proposed that, for cues differing in their learning history and reward associations, the degree of attention they attracted would provide insight into the hedonic value they had recently acquired**.**

As outlined previously, the emotional blink of attention (EBA) task was selected to address this question, based on previous work in the field of eating behaviour. Building on the work of Piech et al. (2010), Davidson et al. (2018) found that the EBA task was sufficiently sensitive to detect fluctuations in attentional capture by palatable foods as a function of an individual's current need state. The authors argued that this measure of attention reflects current implicit motivational salience of a particular food which rises as a meal approaches and hunger rises, but rapidly decreases as the food is eaten to satiety. Consequently, it was predicted that reward learning would modulate the implicit motivational value of specific cues in a similar fashion, and that this technique would allow us to track consequent changes in attention to those cues.

Experiments 1 and 2 (Chapter 3) demonstrated that a food-paired cue (CS+) captured attention to a greater extent than neutral distractors after single trial conditioning. However, an increase in attentional capture was also observed for the CS-, as well as for other related novel shapes, and dessert images, suggesting that each were equally salient, casting doubt on our initial assumption that attentional capture solely reflects the hedonic value of a stimulus. This equivalence raised some important questions about the drivers behind this apparent increase in salience: did

learning transfer, or generalise, from our CS+ to other similar stimuli, or were other factors, such as mere stimulus novelty, at play?

8.3.1 Reward-driven attentional capture and stimulus generalisation

Chapter 3 presents results of a between-subjects experiment in which eating a chocolate geometric object resulted in a significant decrease in performance on trials where either the appetitive CS or a visually similar, unhandled object served as distractor – indicative of enhanced salience. Conversely, for participants who only handled a novel plastic object or a familiar household object, there was no change in attention to their respective stimulus. We propose that this may represent generalisation of reward-driven attentional capture from a food-paired cue, to other similar stimuli.

This finding appears to fall in line with prior research which found that a coloured flanker subsequently captured attention after a singleton of the same colour was paired with a high value reward (Anderson, Laurent & Yantis, 2012), and suggests that stimuli which share some similarity to a reward-paired cue can automatically bias attention despite having no direct reward associations.

Chapter 6 was conducted to explore this phenomenon in more depth. Results revealed a significant pre- to post-conditioning drop in EBA accuracy for target detection on trials where CS+, CS- or two intermediate generalisation stimuli (GS+ and GS-) were presented as distractors, perhaps indicative of stimulus generalisation. These data indicated that each distractor type acquired the same degree of salience across a naturalistic conditioning trial with a CS+ and CS- object – unlike subjective self-reports, which showed a clear generalisation gradient with the strongest responses present for stimuli most similar to a CS+.

These data may represent an adaptive mechanism which serves to maximise the likelihood of securing a homeostatically relevant stimulus. As stimuli sharing some perceptual similarity are likely to be contextually or categorically related, the automatic detection of such stimuli even in the absence of a deliberate search may serve to promote exploration and acquisition of potential reward.

The successful categorization of objects requires the ability to generalise across similar stimuli, but also to discriminate between separate classes (Keller & Schoenfeld, 1950). The ability to categorize accurately is of particular relevance for survival and facilitates facial recognition, correct identification of food, as well as

identification of danger (Troje et al., 1999). The results from Chapter 6 raise the possibility that discrimination is inefficient when newly learned stimuli are presented under conditions of limited awareness. Attempts to maximise discriminability of appetitive stimuli through highly contrasting colours (e.g., purple CS+, yellow CS-), and the use of familiar, easily recognisable shapes resulted in the same pattern of results as those presented in Chapters 3-5, where novel abstract shapes were presented in complementary colours (yellow and orange). Consequently, other possibilities for the unexpected acquisition of salience by unrewarded cues in our naturalistic paradigm must be considered.

8.3.2 Novelty, reward and attention

In uncertain environments, individuals must explore stimuli whose value is currently unknown, in order to exploit the potential resources and opportunities (Dayan & Sejnowski, 1996). A key mechanism thought to motivate such exploration is a bias toward processing and attending to novel stimuli (Krebs et al., 2009). This tendency to investigate novel stimuli is termed the novelty 'exploration bonus' and is considered essential to the survival and success of foraging species (Panksepp, 1998).

Novelty enhances dopaminergic activity in the mid-brain, in a similar way to reward, raising the possibility that reward and novelty are inter-related (Wittman et al., 2007). This pattern of activity encourages the individual to evaluate novel stimuli further, enhancing the possibility of successful reward acquisition. Consequently, novel items are preferentially attended to over familiar ones, even when stimuli are matched in terms of physical salience (Horstmann & Herwig, 2015). Data in Chapters 3-6 may arguably reflect such a mechanism: 3D geometric shapes whose properties were unknown to participants prior to testing consistently captured attention to a greater extent than neutral items after a naturalistic conditioning paradigm, regardless of any associations with reward. It seems possible that uncertainty and surprise surrounding the objects' value and properties heightened their salience to promote further exploration and discovery.

It has been proposed that novel stimuli should lose their capacity to signal reward if they are repeatedly encountered in the absence of reward (Kakade & Dayan, 2002). However, the results presented in Chapter 5 appear to contradict this notion. Despite four naturalistic conditioning trials, both CS+ and CS- stimuli

remained equally salient. The observation that the novelty exploration bonus modulates attention independently of any reward associations suggests that this mechanism is hard wired (Krebs et al., 2009), and may be particularly persistent (Ghazizadeh et al., 2016).

Foley et al. (2014) argue that slow habituation to novel stimuli may be an adaptive mechanism to maximize opportunity for learning. For example, for cues which appear to lack reward associations, it may be beneficial to continue attending to them to address any uncertainty about their properties and avoid missing opportunities for reward. For instance, a particular tree may signal the availability of fruit during summer months but lie dormant during winter. In addition, if a novel stimulus comes to predict a negative outcome, it may be beneficial to explore it further to memorize its attributes and thus avoid it in future. However, despite predictions that reducing novelty of the CS appearance would reduce salience of the non-rewarded cues, Chapter 6 suggests that this was not the case. A familiar shaped CS+ and CS-, as well as two intermediate stimuli, acquired the same degree of salience after conditioning as novel shapes.

The definition of novelty may be of importance here. Bartol, Mirolli & Baldassarre (2013) highlight key differences between novelty and surprise; novelty referring to the frequency with which something has been experienced before, and surprise denoting a stimulus or event which violates expectations somehow. The authors argue that whilst the two terms are typically used interchangeably, it is important to distinguish between them to build a greater understanding of motivated behaviour. Similarly, Ranganath & Rainer (2003) distinguish between stimulus novelty and contextual novelty. Stimulus novelty typically relates to whether a specific stimulus has been experienced before. Conversely, contextual novelty refers to the occurrence of particular stimuli occurring in an unusual or unexpected context. For example, seeing a horse in a field may be a rather ordinary sight, yet seeing a horse in a shopping centre would be much more unusual and more difficult to ignore.

Arguably, the 3D shapes used throughout this thesis could be considered surprising regardless of their appearance; participants were consistently unaware of the objects properties prior to being asked to consume or touch them, and often showed surprise upon discovery. Many participants remarked that the experiments were unusual, and were confused over the possible experimental aims. Furthermore,

the occurrence of these shapes amongst a stream of landscape shapes may be considered out of context, perhaps adding to their attention-grabbing properties.

8.3.3 Reward-independent mechanisms of attentional capture

Whether attentional bias to stimuli associated with reward is acquired in a reward-dependent or reward-independent manner is a matter of controversy, with a number of studies failing to find evidence of reward-dependent mechanisms. Li & Yuhong (2016) found that although participants detected a target associated with high monetary reward faster than those with low or no reward during a training phase, during a test phase attentional processing was equivalent for high, low or no reward. These results arguably stemmed from a difficulty in task switching (Leber & Egeth, 2006); targets which were detected in a training phase subsequently capture attention in test phase regardless of reward associations merely due to recent experience, despite the fact they are no longer relevant to the present task.

Theeuwes (2018) argues that attention is not always the result of top-down or bottom-up processes, but may also be inadvertently guided by selection history. Whilst most research has focused on reward-based selection history, Theeuwes (2013) argues that priming can also bias attention. Through priming, prior experience of a stimulus (e.g., a red target) causes stimuli which share a particular feature (e.g., red distractors) to be processed faster and thus gain prioritised entry into awareness (Theeuwes & Van der Burg, 2013). Again, this facilitation occurs independently of any associations with reward and may provide a much simpler account of why stimuli which share some feature of a CS (e.g., novel geometric CS- shapes) subsequently capture attention.

Regardless of the exact mechanisms at play, this thesis adds to a growing body of evidence suggesting that reward-driven attentional capture may not be entirely value dependent. Instead, our results suggest that attentional capture can be driven by a complex interplay of general attentional mechanisms (see Figure 8.1).

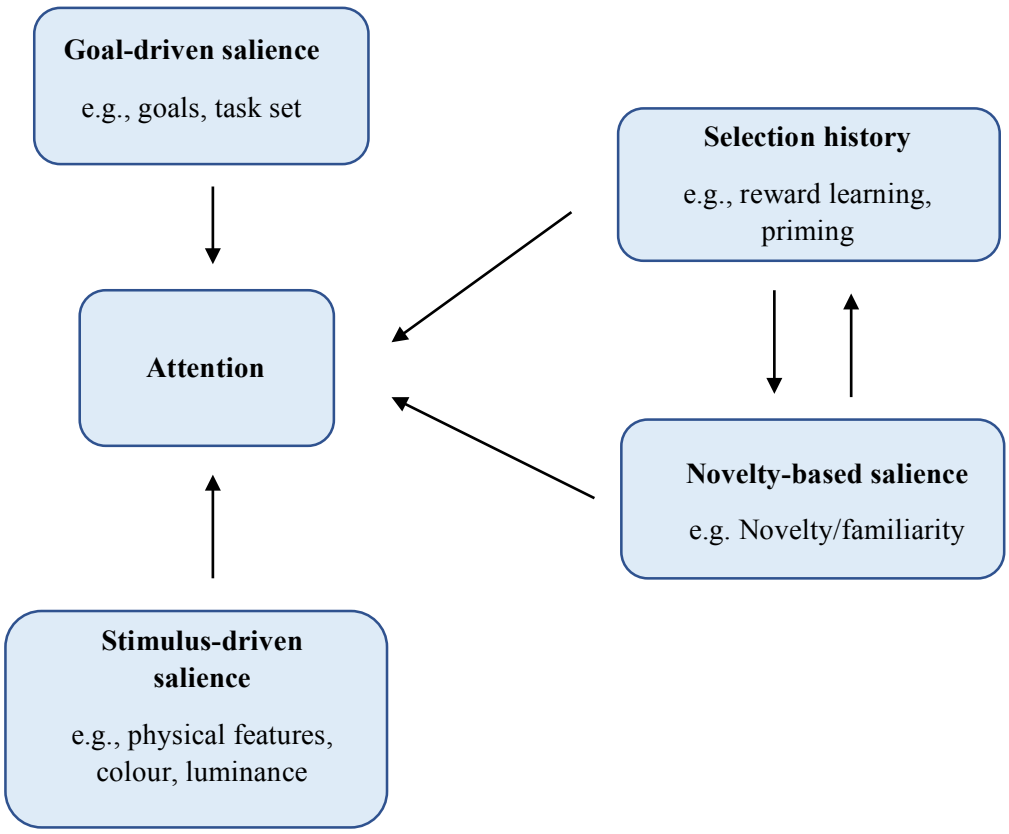


Figure 8.1. A schematic representation of four sources of selection bias and the mechanisms via which they compete for attention. Sources of bias include, the individual's current goal set, physical salience of the stimuli, selection history, including reward associations, and the impact of novelty.

8.4 Neural Underpinnings of Single-Trial Appetitive Conditioning

Experiment 6 (Chapter 7) explored the neural underpinnings of single trial appetitive conditioning during both a passive viewing task and when CS distractors were briefly presented through an EBA task. In an EBA task, neural activity related to attention and salience was greater on CS+ trials than CS- or neutral trials at an uncorrected level, consistent with previous research investigating neural activity during viewing of food and non-food advertisements (Rampuano et al., 2016). In particular, the presence of a CS+ relative to a CS- distractor 200 ms before a target was associated with greater activity in the right insula. Schwabe et al. (2010) found that in a dual-target EBA task, areas of the ventral attention network (VN), including the anterior insula, showed greater activity when identification of a second target was disrupted by the presence of an emotional T1. This suggests that the VN may play an

important role in detecting salient cues in a bottom-up fashion, such as during an EBA task, when responding to such cues is at odds with current goal set.

Similarly, according to Menon & Uddin (2010), the insula forms an important part of the salience network (SN), which overlaps substantially with the VN (Kucyi, Hodaie, & Davis, 2012) and is involved at a pre-attentive level in detecting salient stimuli. It plays a crucial role in attributing salience to the most biologically, homeostatically, motivationally, or cognitively relevant stimulus, and has extensive connections with subcortical nodes which subsequently guide behaviour in an adaptive manner (Menon, 2015). The anterior insula has strong connectivity with the dorsal anterior cingulate cortex (dACC) which plays a more direct role in action selection (Ide, Shenoy, Yu, & Li, 2013). The insula is also known to play a key role in gustatory processing (e.g., Rolls, 2016; Barry et al., 2001), so this activity likely represents the taste of the white chocolate CS.

Similarly, activity in the cuneus was greater during CS+ trials than CS- trials, although again at an uncorrected level. This region of the temporal lobe is thought to be involved in visual processing and can be modulated by reward expectation. Taken together, these data suggest that attentional processing of distractors in the EBA task was guided in a stimulus-driven fashion, and information regarding the taste and reward associated with a CS+ may have informed this process.

In addition, tentative evidence emerged to suggest that CS+ distractors elicited greater activation in the superior and middle temporal gyrus compared to neutral distractors selected from the IAPS database (significant at an uncorrected level).

The superior temporal gyrus is sensitive to the novelty of a stimulus. In oddball paradigms, the STG is more strongly activated by the deviant stimulus (Strobel et al., 2008). The STG is also thought to be involved in the detection of salient stimuli (Downar et al., 2002). The medial temporal lobe has also been shown to be sensitive to novelty (O'Kane, Insler & Wagner, 2015) and is thought to play a key role in recognition memory, particularly object recognition and discrimination (Holdstock, 2005). Consequently, this pattern of activation in the temporal lobe may reflect recognition and effective discrimination of the CS+ compared to neutral distractors, and semantic processing of the object's associated meaning due it its motivational relevance.

These data build on previous chapters by highlighting patterns of neural activity associated with attentional capture by a CS+. Whilst Chapters 3-6 suggest that both CS+ and CS- acquired equal salience, these data hint at effective discrimination of a CS+ relative to a CS-. The involvement of the insula and areas of the temporal lobe may indicate that, in this instance, a greater degree of salience was attributed to a CS+ object made from chocolate than a visually similar plastic CS- or neutral stimuli, perhaps due to its sweet taste. Consequently, CS+ distractors may have 'popped out' more strongly from the RSVP stream. Although, it is important to note that this was the only experiment in this thesis which failed to find differences in target detection accuracy between distractor types on an EBA task (See Chapter 7 for a discussion around the limitations of presenting the EBA task inside the scanner, which may account for the lack of a behavioural effect).

As well as the EBA task, neural activity was also measured during a simple passive viewing task, before and after conditioning. This task was chosen as it is less affected by practice effects and, due to the longer inter stimulus intervals required by fMRI, it is less likely to be affected by the brain's hemodynamic response time. Furthermore, as this task does not require a behavioural response, there is very little interference from motor action and cognitive task demands.

During the passive viewing paradigm, whilst participants simply viewed images of the CS+ and CS-, tentative evidence emerged for a post-conditioning increase in neural activity in the right SFG in response to a CS-. Prior research confirms a role of the right SFG in promoting response inhibition and inhibitory control (e.g., Dambacher et al 2014a; Dambacher et al., 2014b; Hu et al., 2016). In the context of this thesis, the enhanced activity in this region may reflect the brain's capacity to discriminate between related stimuli and prevent an approach response to a low value stimulus that is contextually-linked to the CS+. The modulation of R.SFG activation in response to CS- stimuli in the passive viewing task may reflect the identification of the non-rewarding properties of this stimulus and the consequent prevention of higher level cognitive processing and approach-motivated behaviour. Future research may be useful to further explore the neural mechanisms underlying naturalistic appetitive conditioning using different stimuli under different task constraints.

8.5 Practical Applications and Future Directions

8.5.1 Naturalistic Pavlovian conditioning

Traditionally, Pavlovian conditioning has been studied with CS stimuli that are arbitrary and unrelated to the US. However, Domjan (2005) proposes a more functional approach in which the CS and US must be naturally related. The author argues that such naturalistic conditioning will occur readily under ecologically valid conditions and represents an adaptive trait which promotes survival. In a natural environment, the arbitrary pairing of stimuli is unlikely and would be a rare occurrence. Consequently, the precisely timed and controlled delivery of a US (e.g., sucrose solution) alongside a neutral arbitrary stimulus (e.g., an abstract shape on a computer screen) typical of traditional paradigms does not translate well to natural environments. As a result, the present conditioning paradigm was selected to model a naturalistic encounter with a real food by combining the CS and US.

Domjan et al. (2004) demonstrate that, not only is learning more likely to occur in a naturalistic paradigm, the pattern of learning also differs distinctly between a naturalistic and arbitrary CS. A naturalistic CS appears to facilitate learning and can prevent disruption of the CS-US relationship. For example, they found that a naturalistic CS was resistant to blocking and extinction, and was unaffected by increasing the CS-US interval. Whilst the present thesis hints at a special case of learning for naturalistic conditioning – in this case its rapidity, there is still a considerable requirement for further research to better understand the distinctions between conventional and naturalistic conditioning techniques.

The present paradigm is quick and easy to implement, removing the need for many CS-US pairings. Whilst typical paradigms require careful set-up, and timed delivery to ensure the CS smoothly follows the US, our paradigm means that the CS-US compound simply needs to be presented to the individual, modelling an encounter with a real food. In addition, as shown in Chapters 3 and 6, the CS appearance is relatively easy to modify, and whilst only two different USs were used (chocolate and marzipan), with the advancement of 3D printing technologies the potential for quickly printing edible objects in a wide range of colours, shapes, flavours and ingredients is growing rapidly (Godoi, 2018). As a result, our paradigm provides scope for use in laboratory experiments to answer a wide variety of research

questions, but would also translate easily in to field experiments to better understand how naturalistic conditioning occurs in the real world.

8.5.2 The EBA task

The EBA paradigm has been well established in the fields of cognitive and affective science, proving to be a powerful tool in the study of emotion and attention. This thesis presents one of the first attempts to utilise the EBA method as a measure of incentive salience to explore fluctuations in stimulus value based on experience. The findings of this thesis suggest that the EBA task can serve as a useful measure of stimulus salience; yet, rather than specifically indexing stimulus value as hypothesised, it may better reflect more general mechanisms of attentional prioritization.

Future research may benefit from exploring the sensitivity of the EBA task more fully, using a range of naturally rewarding stimuli (e.g., foods differing in their palatability or attractiveness, or different alcohol related cues), as well as Pavlovian conditioned stimuli. This may provide greater insight into its potential utility for investigating fluctuations in the motivational and attentional processing of different conditioned cues. Furthermore, it may be beneficial for future research to focus on characterizing how salience may guide attention more generally in this paradigm, based on stimulus-driven, goal-driven and experience-driven processes. For example, this thesis highlights a potential role of stimulus and contextual novelty in the automatic attentional capture by otherwise neutral cues, as well as further effects of selection history, such as priming, which have been relatively ignored in the current literature.

This thesis also makes a novel contribution to the field by using fMRI to explore the neural underpinnings of the EBA using reward-paired stimuli for the first time (Chapter 7). Although this did provide some insight, our study highlighted potential challenges with translation of this task for completion inside the scanner using hand held trigger response boxes. Future research may benefit from adjusting and modifying the EBA paradigm, and combining this technique with further neuroimaging methods to better understand the neural mechanisms underlying reward-driven attention and appetitive conditioning.

8.5.3 Implications for treatments and interventions

The present findings have a number of potential implications for tackling issues related to human eating behaviour and obesity. Whilst the present research does not inform us about the long-term implications of reward learning, the fact that just a single trial was sufficient to promote craving and liking, as well as CS-US expectancies, would suggest that in order to minimise the development of food-cue reactivity, interventions need to begin as early as possible in childhood. Preventing experiences with new, calorific products might prove fruitful, for eliminating opportunities for conditioning. Several current policies and interventions are in line with this idea, such as plans to ban the sale of energy drinks to children (Owen, 2018). The potential benefits of total abstinence may at least partially explain the success seen with interventions which ban junk foods in schools (Leonard, 2017); by limiting exposure to such foods, development of food-cue reactivity may be reduced.

Nonetheless, the prohibition of 'unhealthy' food products is a complex issue and it is clear that multiple factors are implicated in obesity, in addition to simple learning processes, which likely all will need to be addressed. Furthermore, whilst complete prohibition is theoretically likely to be effective, it is highly unlikely to be achieved on a grand enough scale to make a significant impact. Every party, holiday, family visit or trip to the supermarket could provide new opportunities for single-trial learning to take place, thus interventions aimed at modifying these associations, once formed, may be more promising.

In addition, appetitive conditioning processes are also receiving greater focus as a potential model for addictions and various psychiatric illnesses (Martin-Soelch et al., 2007). For example, within the addiction literature, Pavlovian conditioning has been heavily implicated in the development of conditioned place preference (CPP; Shalev, Grimm & Shaham, 2002). Drug taking becomes associated with the specific place or context in which it occurred. Consequently, those circumstances will trigger cravings and may lead to relapse when encountered again. Similarly, appetitive conditioning has been implicated in the development and maintenance of eating disorders, such as bulimia and binge eating disorder (Jansen, 1998). Conditioning models of binge eating suggest that consumption of palatable foods serves as a US which elicits powerful physiological changes (URs). Cues such as the sight and smell of food become associated with the US and may begin to elicit powerful CRs such as cravings, triggering a binge. These associations are considered stronger in those with

binge eating disorder (Meule et al., 2018). Consequently, our naturalistic conditioning procedure that effectively mimics a real-life eating episode could provide a useful model for investigating binge eating and compulsive overeating.

Furthermore, a growing literature highlights impairments in response inhibition and salience attribution as playing a key role in drug addiction and abuse. A systematic review by Zilverstand, Huang, Alia-Klein & Goldstein (2018) showed that addicts experience greater activity in the salience network (including the anterior insula) during drug-related processing, but a blunted response during resting states. Chapter 7 highlighted a role for the insula and salience attribution in neural processing of single-trial appetitive conditioning, as well as enhanced activity related to CS- associated inhibitory control in healthy participants. Hence, this technique could provide a basis for further exploring such processes in relation to normal eating behaviour, and in considering whether similar impairments to response inhibition and salience attribution are relevant to eating disorders and obesity.

The fact that in our studies the CS and US form a compound provides a simple paradigm that is quick and easy to implement. This technique could be effectively modified and applied to different controlled laboratory experiments to target a range of research questions. It could also prove valuable in a more ecologically valid research setting. For example, it would be relatively straightforward to adapt this procedure for complex natural environment which provide a range of distractions. Research is increasingly focused on exploring Pavlovian conditioning processes in real-life settings and is beginning to provide greater insights into how human eating behaviour may be influenced by such associative learning in typical daily life (van Den Akker et al., 2018).

Several interventions have been developed with the aim of modifying automatic, impulsive processes in response to food cues, such as attentional bias modification and food-related inhibitory control training. However, given our failure to observe a clear attentional bias for reward-paired cues, it is unclear whether targeting attention in this way would be of benefit. Furthermore, Hardman et al. (2013) found little evidence that attentional biases for food cues could be modified; their attentional bias modification procedure had no effect on hunger or food intake. Current evidence suggests that even when these mechanisms prove successful in the laboratory, they do not translate well to real-world settings (Jones, Hardman,

Lawrence & Field, 2018). Consequently, it may be of more benefit to turn to alternative strategies.

Theoretically, if food-cue reactivity develops via repeated CS-US pairings, then extinction may modify these associations. During extinction, the CS is presented in the absence of the US, so that the CS no longer reliably predicts reward and, consequently, fails to elicit a response (Bouton, 2007). For example, if the sight of a bar of chocolate reliably predicts chocolate consumption, then by going on a diet and repeatedly seeing bars of chocolate without eating them, food-cue reactivity (e.g., cravings and expectancies) should diminish. Although extinction is rather unreliable and difficult to achieve completely (Jansen, Schyns, Bongers & van den Akker, 2016), some therapies attempt to exploit this phenomenon. Cue exposure therapy takes extinction from the laboratory into treatment settings, and aims to tackle cue reactivity, cravings and bingeing amongst eating disordered and obese patients. Although this therapy appears effective in the short term, long term efficacy is more limited (van Den Akker, Schyns & Jansen, 2018). The present conditioning paradigm may offer a novel, more naturalistic approach for the study of extinction and could have important implications for the enhancement of current treatments.

It is important to note that conditioned responses were consistently captured via subjective self-reports, despite difficulties in measuring reward-driven changes on implicit processes. These subjective self-report ratings are thought to reflect motivational processing and are typically associated with P300 and late positive potential (LPP) responses in ERP studies, reflecting a later stage of processing, approximately 300-700 ms after stimulus onset (Preedy, Watson & Martin, 2011). Cognitive reappraisal strategies, aimed at altering the meaning of a response such as food cravings, sufficiently modulated both P300 and LPP amplitude in response to viewing food pictures, at least amongst restrained eaters (Svaldi et al., 2015). Furthermore, similar reappraisal strategies enhanced brain activity in areas related to inhibitory control such as superior frontal gyrus (Yokum & Stice, 2013), and a cognitive appraisal exercise significantly reduced desire to eat highly craved energy-dense foods (Giuliani, Calcott & Berkman, 2013). Consequently, it may prove beneficial to focus interventions on cognitive regulation and reappraisal strategies targeting motivational processing.

It is however, important to note that whilst most people in Western society are bombarded by a plethora of palatable calorific foods and food cues on a daily

basis, many are able to manage their weight and eating behaviour, never becoming obese or developing an eating disorder. Therefore, it may be more useful in future research to explore which individuals are most vulnerable to developing excessive food-cue reactivity and target interventions towards that population. For example, Meule et al. (2018) recently demonstrated that those who experience binges tend to show greater cravings in response to food cues than healthy individuals, perhaps highlighting an enhanced conditionability or sensitivity to appetitive reinforcers in certain individuals. The present conditioning paradigm may offer an efficient and reliable method for exploring these questions.

In addition, Chapter 6 begins to shed light on mechanisms of stimulus generalisation with appetitive stimuli. If such generalisation was to occur excessively, this could theoretically drive a number of disorders related to reward-dysfunction such as obesity and binge eating. It has been shown that excessive fear generalisation is implicated in anxiety and panic disorders (Struyf et al., 2017). For example, Struyf et al. (2017) observed that fear generalisation involves alterations in perception and impaired discrimination between stimuli. Thus, they found a rapid decline in fear responses across a generalisation gradient, depending on the similarity of a generalisation stimulus (GS) to a fear-evoking CS+ or a safe CS-, but individuals with low trait anxiety were more likely to misidentify generalisation stimuli (GS) as the learned safe CS- than those with high trait anxiety. Consequently, the authors argue that further research in to these mechanisms will be beneficial for informing treatments. Similarly, Ehlers and Clarke (2000) proposed the inclusion of perceptual discrimination training in therapeutic interventions for Post-Traumatic Stress Disorder (PTSD). These studies raise the intriguing possibility that similar mechanisms are also involved in appetitive processes and present an intriguing area for future inquiry. Perhaps individuals who are more sensitive to appetitive conditioning will exhibit stronger generalisation to GSs, and may be more likely to respond to stimuli which are relatively dissimilar to the original CS. Indeed, it seems plausible that excessive generalisation with appetitive stimuli could drive reward-related dysfunction such as excessive overeating, drug addiction and pathological gambling.

8.6 Methodological considerations and limitations

8.6.1 Subjective self-reports

The use of subjective self-report measures is commonplace in the appetitive conditioning literature. These single VAS scales offer a valid tool that is quick, easy to implement. However, the potential for bias associated with these measures is worthy of consideration. Response bias in self-report measures can occur for a number of reasons, such as wanting to look good (social desirability) or misunderstanding the question.

In this specific context demand characteristics may have influenced responses, where participants have an awareness of the researchers aims and adjust their responses accordingly (McCambridge, de Bruin & Witton, 2012). For this reason, participants were asked to state their understanding of the aims of each experiment throughout the thesis. Very few participants had an accurate understanding of the research aims and removal of those that did failed to alter the pattern of results. Nonetheless, it is still possible that participants shifted their responses due to a belief that this was expected of them.

Response-shift bias can also pose a challenge in a pre- / post- design, where participants' perceptions and understanding of the measured construct changes from pre-test to post-test (Howard, 1980). It is not possible to say with certainty that participants' perceptions of the questions were the same at both time points, so their ratings may not be solely reflective of the construct to be measured (e.g. cravings).

Future research may benefit from inclusion of alternative measures. For example, retrospective pre-test measures, taken at the same time as post-test measures, have been suggested as a potential adjunct to traditional pre-test- / post-test measures (Drennan & Hyde, 2008).

Furthermore, psychophysiological measures such as heart rate deceleration, facial electromyography (facial EMG), and skin conductance response (SCR) can all provide insight into appetitive conditioned responses, without the same potential for bias. In fact, it may prove particularly important to include a range of measurements in future research as recent findings have highlighted that subjective, physiological and behavioural measures of conditioning may also measure separate constructs (Wardle, Lopez-Gamundi & Flagel, 2018).

8.6.2 Measuring visual attention

An important methodological concern of the current thesis concerns the choice of the EBA task as an indirect assessment of attentional bias by reward-paired cues. In all studies, attentional capture was operationalized as the percentage of trials where a rotated landscape target was correctly identified, when it appeared 200 ms after a distractor image. Poor accuracy reflected greater attentional capture by the distractor. The EBA provides unique insight in to cognitive processing speed and is sensitive to the emotional salience of both positive and negatively valenced affective stimuli (McHugo, Olatunji & Zald, 2013). However, this measure only captures early stages of attentional processing, reflecting initial vigilance. It is unable to capture late stages of processing (reflecting maintenance of attention).

Newer technologies, such as eye-tracking, provide direct measures of visual attention at both early (i.e., initial fixations) and late stages of processing (i.e. last-run dwell times), as well as overall attention cross the whole duration of a stimulus (i.e., total dwell time) (Skinner et al., 2018). Our conclusions are limited by the fact that we only measured responses at short presentation durations (100 ms), which provide an indirect index of early vigilance. Chapters 3-6 suggest that naturalistic objects capture attention automatically after conditioning, regardless of reward associations in this brief window. Measuring responses at longer presentation durations, and in different experimental paradigms may provide important insights in to alternative aspects of reward-driven attention. Perhaps reward-paired cues hold attention for longer than non-reward paired cues. Similarly, if our proposition about novelty and exploration is correct, novel cues with no reward pairings may hold attention for longer as these uncertain cues are investigated. Werthmann (2014) concluded that eye-tracking could provide a more sensitive and reliable measure of visual attention processes than indirect measures and as such may be a valuable addition to future research in this area.

8.6.3 Methodological challenges within differential appetitive conditioning

There are several challenges which make research in to Pavlovian conditioning processes inherently difficult; one of which concerns the repeated presentation of stimuli across experiments (Pastor et al., 2015). As we were investigating the possibility of single-trial learning – which we anticipated would be a rather weak phenomenon, we aimed to maximise the strength of association between the CS and

US, as well as minimise any ambiguities about their associations. With this in mind, although Experiment 1 used a slightly more complex design, incorporating three CS+ and three CS-, Experiments 2-6 presented just a single CS+ and CS-. The fewer the number of CS+ and CS- stimuli, the more each individual stimulus was repeated to ensure adequate power to detect differences on experimental tasks. For example, in Experiments 2-5, the CS stimuli were each presented at least thirty-two times pre- and post-conditioning on the EBA task, in the absence of chocolate consumption.

Multiple presentations of the CS+ and CS- stimuli prior to conditioning may have introduced potential confounds, such as latent inhibition where prior exposure to a stimulus in a neutral context can impair the ability to learn its relevance when paired with an appetitive or aversive outcome (Lubow, 1965). In addition, research suggests that extinction can occur when the CS is presented repeatedly in the absence of the US: a new memory reflecting the now absent CS-US association is formed, and the CS no longer elicits a response (Bouton, 2002). Whilst multiple repetitions of each stimulus type were necessary for these tasks, it is likely that this design choice led to a degree of extinction across the experiment. This possibility is challenged somewhat by the consistently strong evidence for subjective conditioned responses apparent even after multiple stimulus presentations. Additionally, certain subjective responses, such as conditioned likings, may be particularly resistant to extinction (Baeyens, Diaz & Ruiz, 2005). Implicit and psychophysiological measures are perhaps more readily extinguished (Kruse, Tapia León, Stark & Klucken, 2016), which may have masked clear differences from emerging on the EBA task, and could potentially explain the weak evidence for single-trial conditioning at a neural level in Chapter 7.

Furthermore, real-world experiences are unlikely to be limited to such minimal and obvious CS-US pairings. Instead, it is likely that we are subjected to a continuous stream of potential CS-US associations, varying in their form and relevance (Ventura-Bort et al., 2016). For example, if an individual tries a new chocolate bar at a taster stand in a supermarket, will the wrapper alone serve as a CS, or might the chocolate come to be associated with other stimuli such as the time of day, the tray the chocolate is presented on, the person serving the chocolate or the supermarket it is eaten in? In this example, alone, there are hundreds of possible associations and considerable potential ambiguity around the CS-US relationships. Thus, real-world experience may be more effectively represented by utilising more

complex paradigms, to provide a better understanding of the principles underlying reward learning.

Additionally, it is important to note that we used a relatively short fasting period across this thesis (2-3 hours) to ensure participants had not recently eaten. Whilst some studies have demonstrated human appetitive conditioning using food rewards without imposing a fast (Wardle, Lopez-Gamundi & Flagel, 2018), others have shown that conditioning is heightened after a significant period of fasting (approximately 12 hours; Astur, Carew & Deaton, 2014). Future studies might systematically examine the effect of motivational state on appetitive conditioning.

8.6.4 The effect of time on associative learning

In the present thesis, measures of conditioning were taken immediately after conditioning, and were usually completed within ten minutes of the conditioning procedure taking place. Whilst this approach provides us with important information about the immediate effects of our conditioning procedure on motivational and attentional processing, it is unclear whether, or how long these effects would persist.

Within animal research it is commonplace to include longer delays in conditioning paradigms, yet to date, there has been a surprising lack of research in human subjects focusing on the time course and persistence of learning beyond a single short test-session. Evidence from the conditioning literature suggests neural modulation in response to visuo-gustatory processing is apparent up to 24 hours after learning takes place (Viemose et al., 2013), although individuals are likely to encounter much longer delays between CS and US stimuli in the real world. Weeks or months may pass between meals at a favoured restaurant, yet driving past the entrance may elicit powerful memories and desire for a delicious meal.

Wimmer, Li, Gorgolewski & Poldrack (2018) highlight distinct patterns of neural activity between learning which occurred in a single session and learning across well-spaced sessions, with a significant association emerging between working memory and learning. Whilst memory for associations learned in a single-session decayed quickly, lasting memories were formed for associations learned over a number of weeks. It is not possible from the present thesis to infer whether effects of conditioning persisted beyond the isolated test session. Further research is needed to better understand how time influences learning, and how long-lasting associations with reward are formed outside of laboratory settings. A greater focus on the time-

course of learning and the persistence of reward-associations may prove a fruitful area for further study.

8.6.5 Sample size

All studies presented in this thesis had relatively small sample sizes (Ns = 20-40), particularly those with between-subjects procedures (Chapter 4 and 6). These sample sizes were chosen based on typical sample sizes within this field (e.g., Ns = 15-30). Although clear evidence for single-trial appetitive conditioning was observed, it is possible these studies were still relatively underpowered. The use of small sample sizes has been implicated in the apparent 'replication crisis' in the behavioural sciences at present (Button et al.., 2013; Maxwell et al., 2015).

The effects of interest reported by Blechert et al. (2016) and Davidson et al. (2018) were relatively large, (η_p^2 = .197 and .1 respectively). Based on these figures, apriori power calculations, would suggest a sample size of 18 – 34 would be sufficient to establish 80% power for future experiments with a repeated measures design similar to that used in experiments 1 and 2. However, Schäfer & Schwarz, (2019) caution that the publication bias observed in psychology research must be considered when interpreting published effect sizes as these may be inflated in relation to the true population effects..

Using MorePower 6.0 (Campbell & Thompson, 2012), which is particularly usefulfor repeated measures designs, post-hoc power calculations were conducted to examine the degree of power achieved in each of the six experiments presented here. Calculations were conducted given the sample size used, and assuming a conservative medium effect size (η_p^2 = .06), rather than the actual observed effect size for each experiment. Aside from study 4 which achieved adequate power (96%), each of the other experiments were underpowered (21% - 79%), suggesting that lack of power may account for some of the findings, or lack of, presented in this thesis.

Nonetheless, the practice of conducting power calculations after an experiment is complete is fundamentally flawed and can be misleading (Hoenig & Heisey, 2012; Gilbert & Prion, 2016) and as such, these calculations should be interpreted with caution. Whilst it is likely that these experiments were underpowered, this does not give sufficient grounds to conclude that stronger effects would have been observed with larger sample sizes. Researchers should ensure

future studies are pre-registered and well powered; apriori power analyses should be conducted in advance of the experiment to allow more certainty.

8.6.6 Individual differences

Previous studies have suggested an important role of individual differences on incentive-motivational processes related to food and food related cues, yet to date these are not well understood. For example, evidence suggests that overweight participants demonstrate greater conditioned responding to neutral cues paired with hedonic food stimuli (Meyer et al., 2015), whereas other studies have suggested an impairment in acquisition of conditioned responses by obese women (Zhang, Manson, Schiller & Levy, 2014). It has also been argued that obese women may over-generalise learning from CS+ to CS- stimuli (Van Den Akker et al., 2018).

Furthermore, incentive salience for palatable food cues may be higher for obese individuals: a greater attentional bias for palatable food cues has been observed amongst obese, compared to lean individuals (Doolan et al., 2014), although the opposite pattern has also been observed (Nummenmaa et al., 2011). Personality traits, such as impulsivity and external eating may also influence the degree to which attentional processes are biased towards rewarding stimuli (Coskunpinar & Cyders, 2013; Hou et al., 2011). Consequently, it may be necessary to isolate potential confounds and apply stringent controls in order to uncover genuine effects.

Different eating-related traits, including external eating, emotional eating, dietary restraint and food neophobia were measured through Chapters 3-7, and reward sensitivity was measured through Chapters 4-7. There appeared to be no clear or consistent associations between these traits and single trial conditioning processes, but sample sizes were small and there was not a great enough spread of scores to warrant analysis based on separate groups. A more thorough exploration of the impact of such individual differences may be an important area of future study. However, it should be noted that a recent study by Kirsten et al., (2019) which specifically explored these issues found no relationship between attentional capture by food stimuli in a RSVP task and restrained, emotional or external eating style.

8.7 Concluding remarks

In summary, this thesis examined the relationships between learning, motivational and attentional processing, using implicit and explicit measures. A primary finding was that, with a US of sufficient value, single-trial appetitive conditioning is a potent phenomenon in humans. Subjective measures of conditioning (craving, liking and US-expectancy) can be learned rapidly, after just one naturalistic encounter with a 'new' food, which has important implications for understanding food-cue reactivity in humans, and developing interventions to tackle hedonic overeating. We demonstrated for the first time that this reward learning can generalise to related stimuli in proportion to their perceptual similarity to the CS+. Of particular interest was the finding that multiple trials did little to strengthen CS-US associations; the greatest learning occurred in a single-trial, with little evidence of any further learning beyond this point. It was also predicted that using single-trial appetitive conditioning, attention would be mediated by the incentive salience of newly learned stimuli. However, a consistent finding was that salience increased generally for any stimulus sharing some perceptual similarity to the CS+, or that was contextually linked to the conditioning procedure. We propose that whilst attention may be mediated by incentive salience, under certain circumstances – particularly where there is uncertainty about a stimulus' properties, novel or unusual stimuli may be selected for further processing to promote further exploration and discovery. Further characterizing the mechanisms underlying the learning of stimulus value across multiple tasks and types of reward may prove essential for better understanding the development of real-world, motivationally-relevant attentional biases. It is hoped that the findings of this thesis provide the groundwork for future research in to the subjective, attentional and neural processes underpinning rapid appetitive conditioning and its subsequent impact and relevance for human eating behaviour.

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Appendices

The following appendices contain descriptive, methodological and analytic information, which is considered supplementary to the main thesis and is presented here for completeness and additional clarity.

Appendix A

Participant Number:

UNIVERSITY OF LIVERPOOL

Food Liking Questionnaire

This questionnaire is designed to assess your liking/disliking of various foods.

For each food item, please check the box that best expresses your opinion of that food. It is important that you answer as accurately as possible as you may be asked to eat items containing or more of these foods in the main experiment. Please check no more than one box for each of the 10 food items.

Here is a completed example,

Food Items	Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
Cake								X	

Now, please complete the following questions. Please ensure you have provided an answer for every food item.

Food Items	Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
Pasta									
Crisps									
Chocolate									
Carrots									
Marzipan									
Apples									
Custard									
Tomatoes									
Cheese									
Meringue									

Please return the completed questionnaire to the researcher who will then inform you as to whether you are eligible to take part in the main experiment.

Appendix B

Medical History Questionnaire.

Participant Number _____

This questionnaire is designed to establish your suitability for this research project. The questionnaire will not be used as part of the study data but will be kept separately and securely for your wellbeing during the study.

Some of the questions ask about personal information. If you do not wish to answer please let the researcher know. All information taken is confidential.

Please answer as honestly as possible.

1. Are you taking or using any medicine or any other drug, either from your doctor or of your own accord? If so, please list the items below: **Yes / No**

_____

2. Are there any foods you don't eat? If so, please state what and why. **Yes / No**

_____

3. Are you allergic to anything that you are aware of? **Yes / No**

The following foods have been known to cause allergies. Have you ever consumed these foods **AND** had an allergic reaction to them?

	Previously Consumed	Allergic Reaction
Peanuts	**Yes / No**	**Yes / No**
Nuts	**Yes / No**	**Yes / No**
Almonds	**Yes / No**	**Yes / No**
Marzipan	**Yes / No**	**Yes / No**
Dairy produce	**Yes / No**	**Yes / No**
Seeds	**Yes / No**	**Yes / No**
Eggs	**Yes / No**	**Yes / No**
Fish	**Yes / No**	**Yes / No**
Shellfish	**Yes / No**	**Yes / No**
Soy(a)	**Yes / No**	**Yes / No**
Celery	**Yes / No**	**Yes / No**
Mustard	**Yes / No**	**Yes / No**
Strawberries	**Yes / No**	**Yes / No**

	Previously Consumed	Allergic Reaction
Cherries	**Yes / No**	**Yes / No**
Kiwifruit	**Yes / No**	**Yes / No**
Pulses	**Yes / No**	**Yes / No**
Foods containing **sulphur dioxide/sulphites**	**Yes / No**	**Yes / No**
Foods containing **lupin**	**Yes / No**	**Yes / No**
Foods containing **gluten**	**Yes / No**	**Yes / No**
Foods containing **lactose**	**Yes / No**	**Yes / No**
Foods containing **wheat**	**Yes / No**	**Yes / No**

4. Are there any foods which make your mouth, lips or throat tingle?
Yes/No

5. Have you ever suffered from anaphylaxis or anaphylactic shock?
Yes/No

6. Did you suffer from severe childhood allergies?
Yes/No

For Office use only

Date Screened	**All questions answered**	**Yes / No**
Name of Researcher	**Suitable for study**	**Yes / No**

Appendix C

This appendix concerns the correlation matrix between task, self-report measures and measures of individual differences for two experiments presented in Chapter 3: experiment 1 (Table C1) and experiment 2 (Table C2).

This additional analysis was concerned with whether responsivity to single-trial appetitive conditioning was related to relevant individual differences. Difference scores for EBA task performance and self-report measures of conditioning were computed by subtracting the pre-conditioning scores from the post-conditioning scores. A Holm-Bonferroni sequential correction was carried out to correct for multiple comparisons (Holm, 1979). In the standard Bonferroni correction, the alpha level (α) is divided by the number of hypotheses tested (*n*). However, the Holm-Bonferroni involves calculating the p-values for all tests and ordering them sequentially by rank (i.e. the smallest p-value is assigned rank 1). P-values are then corrected by applying the following formula, $\alpha / (n - \text{rank} + 1)$. If $p < \alpha$ then the null is rejected and the formula is applied to the next ranked p-value. If $p > \alpha$, the null hypotheses is accepted for all remaining tests and testing stops. This test is less conservative than the standard Bonferroni correction and thus has greater power (Chen, Feng & Yi, 2017).

Table C1 Pearson correlations between pre- to post-conditioning difference scores on attentional capture (EBA % CRs), self-report measures (valence ratings and cravings) and individual differences (FNS, DEBQ).

	Attention				**Self-report**					
	% Correct responses (EBA)				Valence (VAS)					Cravings
	Pre-post (CS+)	Pre-post (CS-)	Pre-post (Novel)	Pre-post (Neutral)	Pre-post (CS+)	Pre-post (CS-)	Pre-post (Novel)	Pre-post (Neutral)	Pre-post (Filler)	Pre-post
BMI	.24	-.01	-.19	.02	.10	.03	-.11	-.01	-.09	-.09
Food Neophobia										
FNS: Total	-.12	.07	.23	-.01	.08	-.12	-.24	-.11	-.15	.27
Eating Styles										
DEBQ: Restraint	-.01	-.15	-.02	.04	-.04	.18	.26	-.10	-.24	-.05
DEBQ: Emotional	.02	-.08	.21	.22	-.16	-.19	-.03	.05	.22	-.09
DEBQ: External	.16	-.17	.23	-.01	.21	-.03	.14	-.16	.11	-.28

*Significant at corrected alpha. †Significant at the level of the uncorrected alpha. All two-tailed. *EBA* = Emotional blink of attention (% correct responses); *Pre-post (CS+)* = change scores for CS+ stimuli; *Pre-post (CS-)* = change scores for CS- stimuli; *Pre-post (Novel)* = change scores for Novel stimuli; *Pre-post (Neutral)* = change scores for Neutral stimuli; *BMI* = body mass index; *FNS* = Food Neophobia Scale; *DEBQ* = Dutch Eating Behaviour Questionnaire

Table C2 Pearson correlations between pre- to post-conditioning difference scores on attentional capture (EBA % CRs), self-report measures (valence ratings and cravings) and individual differences (FNS, DEBQ).

	Attention				**Self-report**					
	% Correct responses (EBA				Valence (VAS)					Cravings
	Pre-post (CS+)	Pre-post (CS-)	Pre-post (Dessert)	Pre-post (Neutral)	Pre-post (CS+)	Pre-post (CS-)	Pre-post (Dessert)	Pre-post (Neutral)	Pre-post (filler)	Pre-post
BMI	-.13	.05	-.08	-.01	-.24	-.13	-.02	.01	.11	.14
Food Neophobia										
FNS: Total	.05	.21	-.07	.13	-.12	-.06	.15	**-.44†**	.11	.06
Eating Styles										
DEBQ: Restraint	.02	.03	-.01	.16	-.06	-.15	-.06	-.18	.18	.24
DEBQ: Emotional	.06	.04	-.12	-.05	-.06	-.14	.28	.25	.20	.06
DEBQ: External	-.03	-.28	.14	-.01	-.03	.04	.07	.24	.04	-.19

*Significant at corrected alpha. †Significant at the level of the uncorrected alpha. All two-tailed. *EBA* = Emotional blink of attention (% correct responses); *Pre-post (CS+)* = change scores for CS+ stimuli; *Pre-post (CS-)* = change scores for CS- stimuli; *Pre-post (Dessert)* = change scores for Dessert stimuli; *Pre-post (Neutral)* = change scores for Neutral stimuli; *BMI* = body mass index; *FNS* = Food Neophobia Scale; *DEBQ* = Dutch Eating Behaviour Questionnaire.

Appendix D

This appendix concerns Experiment 3 presented in Chapter 4, Table D1 presents average familiarity ratings of a random selection of experimental stimuli before and after conditioning/exposure to an appetitive, novel or familiar stimulus. Table D2 presents the average valence ratings (pleasantness) of the same stimuli. Finally, Table D3 concerns the correlation matrix between task, self-report measures and measures of individual differences. As predicted, conditioned responses were only evident for participants in an appetitive CS condition, thus, correlations were only calculated between individual differences and outcome measures for this condition only.

Table D1. Mean (SD) familiarity ratings of experimental stimuli pre- and post-exposure to an appetitive, novel or familiar object.

	CS Mean (*SD*)		**Novel Stimulus** Mean (*SD*)		**Familiar Stimulus** Mean (*SD*)		**Neutral** Mean (*SD*)		**Landscape Fillers** Mean (*SD*)	
Condition	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Appetitive CS	55.32(*11.91*)	54.13(*8.07*)	56.84(*11.11*)	48.98(*9.78*)	52.09(*11.27*)	49.89(*10.38*)	53.38(*8.91*)	53.31(*9.34*)	53.72(*9.45*)	55.24(*9.07*)
Novel CS	55.79(*15.17*)	54.55(*12.92*)	53.60(*12.88*)	53.62(*12.79*)	53.50(*13.13*)	57.68(*9.95*)	53.29(*11.36*)	49.56(*11.29*)	54.37(*12.43*)	50.86(*13.72*)
Familiar Stimulus	53.66(*13.10*)	53.35(*12.17*)	49.70(*12.51*)	53.08(*13.35*)	51.66(*13.12*)	51.85(*8.45*)	51.63(*10.39*)	54.43(*11.54*)	50.14(*11.59*)	51.35(*10.81*)

Table D2. Mean (SD) valence ratings of experimental stimuli pre- and post-exposure to an appetitive, novel or familiar object.

	CS Mean (*SD*)		Novel Stimulus Mean (*SD*)		Familiar Stimulus Mean (*SD*)		Neutral Mean (*SD*)		Landscape Fillers Mean (*SD*)	
Condition	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Appetitive CS	51.50(*11.99*)	54.43(*12.20*)	54.41(*11.44*)	52.88(*11.81*)	53.70(*9.70*)	50.99(10.63)	53.47(*10.15*)	53.98(9.99)	56.54(*9.89*)	55.35(10.87)
Novel CS	52.88(*12.07*)	52.58(*13.21*)	56.62(*6.94*)	54.58(*10.63*)	54.16(*13.57*)	52.09(14.70)	54.13(*13.25*)	53.08(13.02)	52.48(*11.16*)	53.19(9.93)
Familiar CS	52.35(*14.21*)	48.98(*13.31*)	52.17(*10.99*)	53.58(*10.58*)	52.06(*10.34*)	53.61(16.12)	53.85(*13.07*)	52.05(13.78)	53.73(*10.55*)	48.75(12.33)

Table D3 Pearson correlations between individual differences (FNS, DEBQ, BAS-RR, BSSS) and outcome measures (pre- to post-conditioning difference scores on attentional capture (EBA % CRs) and self-report measures (cravings and expectancies) for the appetitive CS condition.

	Attention				**Self-report**	
	% Correct responses (EBA)				Cravings	Expectancies
	Pre-post (CS)	Pre-post (Novel)	Pre-post (Neutral)	Pre-post (Familiar)	Pre-post	Pre-post
BMI	-.25	-.02	-.18	-.18	.19	.16
Food Neophobia						
FNS: Total	-.07	.04	-.21	-.05	.13	.05
Eating Styles						
DEBQ: Restraint	-.07	.03	.03	-.12	.19	.22
DEBQ: Emotional	-.24	.19	-.02	-.07	-.10	-.03
DEBQ: External	-.18	.10	-.14	-.08	-.27	-.32
Reward Sensitivity						
BAS-RR	.37	-.01	.18	-.10	.27	.05
BSSS-Total	.12	-.30	**.46 †**	.18	-.08	.39

*Significant at corrected alpha. †Significant at the level of the uncorrected alpha. All two-tailed. EBA = Emotional blink of attention (% correct responses); Pre-post (CS) = change scores for an appetitive CS stimuli (chocolate 3D geometric object); Pre-post (Novel) = change scores for novel stimuli (novel 3D geometric shape); Pre-post (Neutral) = change scores for IAPS neutral stimuli; Pre-post (Familiar) = change scores for familiar stimuli (pencil); BMI = body mass index; FNS = Food Neophobia Scale; DEBQ = Dutch Eating Behaviour Questionnaire. BAS-RR = Behavioural activation system – Reward responsivity; Brief-SSS = Brief Sensation Seeking Scale.

Appendix E

This appendix concerns the correlation matrix between task, self-report measures and measures of individual differences for experiment 4 presented in Chapter 5 (Table E1).

Table E1 Pearson correlations between individual differences (FNS, DEBQ, BAS-RR, BSSS) and outcome measures (pre- to post-conditioning difference scores on attentional capture (EBA % CRs) and self-report measures (cravings, expectancies and liking) for the CS+.

	Attention				**Self-report**		
	% Correct responses (EBA)				Cravings	US-Expectancies	Liking
	Pre-post (CS+)	Pre-post (CS-)	Pre-post (Dessert)	Pre-post (Neutral)	Pre-post	Pre-post	Pre-post
BMI	.07	<.01	.04	.30	.08	**.34†**	.13
Food Neophobia							
FNS: Total	**.35†**	.24	-.11	.32	.30	.22	-.17
Eating Styles							
DEBQ: Restraint	.14	-.08	.11	.30	-.04	.11	.27
DEBQ: Emotional	-.01	-.07	-.26	.02	.14	.30	-.18
DEBQ: External	.04	-.15	.01	-.02	-.09	-.26	.09
Reward Sensitivity							
BAS-RR	-.20	-.16	-.12	-.21	-.12	-.22	-.16
BSSS-Total	-.22	-.22	-.19	**-.33†**	-.16	.01	-.25

*Significant at corrected alpha. †Significant at the level of the uncorrected alpha. All two-tailed. EBA = Emotional blink of attention (% correct responses); Pre-post (CS+) = change scores for a CS+; Pre-post (CS-) = change scores for CS-; Pre-post (Dessert) = change scores for dessert images; Pre-post (Neutral) = change scores for IAPS neutral stimuli; BMI = body mass index; FNS = Food Neophobia Scale; DEBQ = Dutch Eating Behaviour Questionnaire. BAS-RR = Behavioural activation system – Reward responsivity; Brief-SSS = Brief Sensation Seeking Scale.

Appendix F

This appendix concerns Experiment 5 (Chapter 6). It presents ANOVA and t-test analyses for exploring the effect of time point (pre- and post-conditioning) and distractor type (CS+, GS+, GS-, CS- and neutral) on EBA task performance (% accuracy). Although this analysis was originally planned in line with our aims and hypotheses, it is presented in the appendix rather than the main text due to the fact that main effect and interactions with condition failed to reach significance in a 2 × 2 × 5 mixed ANOVA (See Chapter 6). This follow up analysis is included here for completeness. A correlation matrix is also presented concerning associations between EBA task performance, self-report measures of conditioning and individual differences.

Exploring of the effect of condition on attentional capture by CS, GS and neutral stimuli.

Data was split by condition and a 2 (time point) × 5 (distractor type) ANOVA was conducted. This revealed that for both conditions, the main effect of time, main effect of type and the interaction term were significant ($ps \leq .007$).

Further to this, the simple effect of time-point was explored for each distractor type with a series of paired t-tests (See Table F.1). This revealed that accuracy on CS+, CS- and GS+ distractor trials decreased from pre- to post-conditioning across both conditions. Similarly, accuracy remained unchanged for neutral distractors in both conditions. However, whilst accuracy on GS- distractor trials decreased for participants in the Novel condition, accuracy on GS- trials remained unchanged in the Familiar stimulus condition.

Examination of the means would indicate that this effect is likely driven by slightly lower attentional capture by GS- cues at baseline in the familiar condition than novel. As the CS and GS stimuli were visually similar, matched for luminance and had no prior reward associations for participants, this is most likely a chance finding.

Table F.1 Pairwise Comparisons of Average Target Detection Accuracy pre- and post-conditioning for each distractor type, split by condition (familiar or novel).

		% Correct Responses Mean ± SD		Statistics		
Condition	Distractor type	Pre-conditioning	Post-conditioning	t-value	*p-value*	***d***
Familiar	CS+	73.56 (13.09)	63.58 (16.70)	4.41	< .001	.87
	GS+	68.75 (15.59)	57.81 (17.19)	3.83	.001	.75
	GS-	66.11 (15.12)	62.38 (16.36)	1.26	.219	.25
	CS-	71.64 (14.60)	64.78 (14.32)	2.92	.007	.57
	Neutral	70.79 (13.14)	70.31 (12.03)	.25	.804	.05
Novel	CS+	72.69 (14.78)	61.82 (15.10)	4.58	< .001	.96
	GS+	70.52 (14.65)	62.23 (17.37)	5.02	< .001	1.05
	GS-	70.79 (14.71)	61.55 (17.54)	3.07	.006	.64
	CS-	72.01 (12.97)	60.33 (17.69)	3.88	.001	.81
	Neutral	74.32 (12.60)	74.32 (13.59)	.00	1.00	0

Note: Familiar df = 25, Novel df = 22.

Associations with individual differences

Table F.2 Pearson correlations between individual differences (FNS, DEBQ, BAS-RR, BSSS) and outcome measures (pre- to post-conditioning difference scores on attentional capture (EBA % CRs) for CS+, GS+, GS-, CS- and Neutral distractor trials, as well as self-report measures (cravings, expectancies and liking) for the CS+.

	Attention					**Self-report**		
	% Correct responses (EBA)					Cravings	US-Expectancies	Liking
	Pre-post (CS+)	Pre-post (GS+)	Pre-post (GS-)	Pre-post (CS-)	Pre-post (Neutral)	Pre-post	Pre-post	Pre-post
BMI	.19	-.14	<-.01	.01	.04	-.10	.01	**-.37†**
Food Neophobia								
FNS: Total	-.01	.12	-.01	.20	.11	.02	.02	-.09
Eating Styles								
DEBQ: Restraint	.08	-.05	**-.30†**	-.05	-.07	-.01	-.01	.08
DEBQ: Emotional	-.10	.01	-.09	-.07	-.13	.04	-.05	-.08
DEBQ: External	-.02	-.08	.02	.06	-.14	-.06	.04	.18
Incentive sensitization								
BAS-RR	.01	-.08	.15	.02	.10	-.03	.17	**.41***
BSSS-Total	.12	.02	.02	.02	-.06	-.05	.18	.02

*Significant at corrected alpha. †Significant at the level of the uncorrected alpha. All two-tailed. EBA = Emotional blink of attention (% correct responses); Pre-post (CS+) = change scores for a CS+; Pre-post (CS-) = change scores for CS-; Pre-post (GS+) = change scores for GS+; Pre-post (GS-) = change scores for GS-Pre-post (Neutral) = change scores for IAPS neutral stimuli; BMI = body mass index; FNS = Food Neophobia Scale; DEBQ = Dutch Eating Behaviour Questionnaire. BAS-RR = Behavioural activation system – Reward responsivity; Brief-SSS = Brief Sensation Seeking Scale.

Appendix G

This appendix concerns Experiment 6 (Chapter 7). It presents MNI coordinates for apriori regions of interest used to conduct an ROI analysis exploring BOLD activity at each ROI during a passive viewing paradigm and an EBA task involving presentation of CS+ and CS- stimuli (Table G1). It also presents a correlation matrix between voxel signal change (pre- to post-conditioning) in the R.SFG during a passive viewing task, self-report measures and measures of individual differences (Table G2). Finally, it presents a correlation matrix between BOLD signal during an EBA task, self-report measures of conditioning and individual differences.

Table G1 *A priori* regions of interest and their coordinates.

Anatomical location	MNI coordinates
Left amygdala	-22 -4 -16
Right amygdala	30 0 -22
Left nucleus accumbens (NAcc)	-12 10 -8
Right NAcc	12 10 -8
Orbitofrontal cortex (OFC)	2 58 -8
Anterior cingulate cortex (ACC)	2 32 16
Caudate tail	36 -43 -2
Lateral occipital complex (LOC)	-52 -70 -6
Intraparietal sulcus (IPS)	-36 -57 50
Extrastriate cortex	-30 -86 24

MNI = Montreal Neurological Institute.

Table G2 Pearson correlations between change in BOLD signal at peak voxels in the R.SFG in response to a CS+ and CS- pre- and post-conditioning during a passive viewing paradigm, individual differences (FNS, DEBQ, BAS-RR, BSSS) and self-report outcome measures (pre- to post-conditioning difference scores (cravings, expectancies and liking) for a CS+ and CS-.

Brain Activity		**Self-report measures (CRs)**						**Individual Differences**						
Anatomical Location	Voxel Change	CS+ Crave Post-Pre	CS+ Expect Post-Pre	CS+ Liking Post-Pre	CS- Crave Post-Pre	CS- Expect Post-Pre	CS- Liking Post-Pre	BMI	FNS	DEBQ-R	DEBQ-Em	DEBQ-Ex	B-SSS	BAS-RR
R.SFG	CS+	.24	**.45†**	.34	-.03	.12	.05	<-.01	.09	.02	.17	.16	-.14	-.07
	CS-	.39	-.12	.20	-.12	-.08	.14	-.18	<-.01	.04	.15	.24	.43	.21

*Significant at corrected alpha. †Significant at the level of the uncorrected alpha. All two-tailed. R.SFG = Right superior frontal gyrus. BMI = body mass index; FNS = Food Neophobia Scale; DEBQ = Dutch Eating Behaviour Questionnaire. BAS-RR = Behavioural activation system – Reward responsivity; Brief-SSS = Brief Sensation Seeking scale

Table G3 Pearson correlations between brain activity during an EBA task , individual differences (FNS, DEBQ, BAS-RR, BSSS) and self-report outcome measures (pre- to post-conditioning difference scores (cravings, expectancies and liking) for a CS+ and CS-.

Brain Activity		**Self-report measures (CRs)**						**Individual Differences**						
Contrast	Anatomical Location	CS+ Crave Post-Pre	CS+ Expect Post-Pre	CS+ Liking Post-Pre	CS- Crave Post-Pre	CS- Expect Post-Pre	CS- Liking Post-Pre	BMI	FNS	DEBQ-R	DEBQ-Em	DEBQ-Ex	B-SSS	BAS-RR
CS+ > CS-														
	R.Insula	.27	-.17	<.001	-.38	-.18	.12	.47	.12	.08	-.36	-.30	**.48**†	-.07
	L.Cuneus	.18	.16	.31	-.04	-.01	-.25	.09	.18	.14	-.22	-.20	.06	-.30
CS+ > Neutral														
	L.STG	.37	.26	.10	**-.48**†	-.37	.21	.43	.21	-.18	-.23	-.14	.43†	.22
	L.MTG	-.01	.15	-.01	-.21	-.31	.30	.26	.37	-.39	.09	-.10	.32	.26

*Significant at corrected alpha . †Significant at the level of the uncorrected alpha. All two-tailed. R = Right; L = Left; STG = superior temporal gyrus; MTG = Medial temporal gyrus; BMI = body mass index; FNS = Food Neophobia Scale; DEBQ = Dutch Eating Behaviour Questionnaire. BAS-RR = Behavioural activation system – Reward responsivity; Brief-SSS = Brief Sensation Seeking Scale.